Design, synthesis and testing of β-strand mimics as protease inhibitors

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Abstract

Chapter 1 gives background information on proteases and discusses the concept of protease inhibition as a therapeutic strategy for humans. It introduces the key concept that conformation defines biological activity. It also outlines how proteases almost universally bind their substrate/inhibitors in an extended β-strand conformation. The use of calpain as a prototype protease for the testing of β-strand mimics synthesised later in the thesis is also discussed.

Chapter 2 describes how molecular modeling was used to rationalise the structure based activity relationships (SAR) of known calpain inhibitors. Molecular modeling was then used to successfully design a number of acyclic β-strand mimics. The synthesis and testing of eight such inhibitors is described. The most potent β-strand mimic prepared was **2.13**. This was determined to have an IC_{50} of 30 nM against calpain II.

Chapter 3 outlines the history and application of ring closing metathesis (RCM) to the synthesis of cyclic compounds. The attempted synthesis of an eight membered cyclic nitrogen to nitrogen conformationally constrained dipeptide is described. The synthesis of a conformationally constrained β-amino acid calpain inhibitor (**3.73)** is also described.

A novel calpain inhibitor motif was designed in **Chapter 4.** On the basis of this an *in-silico* combinatorial library of two hundred and eighty eight possible β-strand templates was prepared. Conformational analysis of this library was performed and from this a number of excellent β-strand templates were identified and selected for synthesis. The preparation of ten β-strand templates is described. New microwave irradiation methodology was developed to achieve this.

The formation of a six-membered catalyst deactivating chelate is also proposed to explain why some dienes fail to undergo RCM. Two methods to circumvent the formation of such a chelate are outlined. The addition of Lewis acid chloro-dicyclohexyl borane to the RCM reaction mixture and chain length alteration are investigated.

Chapter 5 describes the design of macrocyclic β-strand mimics using induced fit molecular modelling. The physicochemical properties of these were calculated *in-silico*. From this analysis a number of Tyr-XX-Gly based and Tyr-XX-Cys based macrocyclic calpain inhibitors were selected for synthesis. The preparation and testing of these are described. In the Tyr-XX-Gly macrocyclic system a number of variables were investigated and numerous SAR implications concluded. Aldehyde **5.14** was identified as the best electrophilic warhead macrocyclic calpain inhibitor with an IC_{50} against calpain II of 27 nM. The best non-electrophilic warhead macrocycle (**5.13**) had an IC_{50} against calpain II of 704 nM.

Chapter 6 describes synthetic optimisation for the preparation of calpain inhibitors **2.13**, **5.14** and **5.17.** Multi-gram quantities of each were prepared. Aldehydes **2.13** and **5.14** were evaluated as anti-cataract agents using *in-vivo* cataract sheep model. Both of these β-strand mimics were demonstrated to retard cataract development. Macrocycle **5.14** was found to be the most effective, decreasing the rate of cataract development between forty four and forty nine per cent relative to control.

Chapter 7 outlines the attempted development of RCM methodology for the chiral synthesis of α-α disubstituted amino acid lactams. In addition, methodology for the stereoselective incorporation of a C-N constrained β-amino acid carbocycle into a peptide or peptidomimetic is described.

ABBREVIATIONS

1,1,2-TCE	1,1,2-Trichloroethane
δ	Chemical shift
BOC	*tert*-butoxycarbonyl
BODIPY	4,4-difluoro-5,7-dimethyl-4-bora-3a,4-diaza-*s*-indacene-3-propionic acid
bs	Broad singlet (in NMR)
CBZ	Benzyloxycarbonyl
COSY	Correlation spectroscopy
cy	Cyclohexane
d	Doublet (in NMR)
DCM	Dichloromethane
dd	Doublet of doublets (in NMR)
ddd	Doublet of doublets of doublets (in NMR)
dddd	Doublet of doublets of doublets of doublets (in NMR)
dt	Doublets of triplets (in NMR)
DIBAL-H	Diisobutylaluminium hydride
DIPEA	N,N-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	N,N-dimethylforamide
EtOAc	Ethyl acetate
EDC	1-[3-(dimethlamino)propyl]-3-carbodiimide hydrochloride
DMSO	Dimethyl sulfoxide
EI	Electron impact ionization (in mass spectrometry)
equiv	Equivalent(s)
ES	Electrospray ionization
Et_2O	Diethyl ether
FTIR	Fourier transform infrared
Grubbs first generation catalyst	Benzylidene-bis(tricyclohexylphosphine)dichlororuthenium
Grubbs second generation catalyst	Benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium.

HATU	N,N,N',N'-Tetramethyl-O-(7-azabenzotriazol-1-yl)uranium hexafluorophosphate
HCl	Hydrogen chloride
$HCl_{(aq)}$	Hydrochloric acid
HMBC	Heteronuclear multiple bond correlation (in NMR)
HOAt	1-Hydroxyazabenzotriazole
HOBt	1-Hydroxybenzotriazole
HRMS	High resolution mass spectrometry
h(s)	Hour(s)
HSQC	Heteronuclear single quantum correlation (in NMR)
Hz	Hertz (in NMR)
KOH	Potassium hydroxide
J	Coupling constant
K_2CO_3	Potassium carbonate
LCMS	Liquid chromatography mass spectrometry
LDA	Lithium diisopropylamide
LiHMDS	Lithium bis(trimethylsilyl)amide
$LiAlH_4$	Lithium aluminium hydride
LRMS	Low resolution mass spectrometry
m	Multiplet (in NMR)
MeOH	Methanol
min(s)	Minute(s)
$MgSO_4$	Magnesium sulphate
mp	Melting point
NaOH	Sodium hydroxide
NH_4Cl	Ammonium chloride
NMR	Nuclear magnetic resonance
Pet ether	Petroleum ether (50-70^o C)
Pd/C	Palladium on carbon catalyst
ppm	Parts per million
PyAOP	(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
RCM	Ring closing metathesis
RT	Room temperature
SAR	Structure activity relationship
s	Singlet (in NMR)

SO_2Cl	Thionyl chloride
SO_3.Pyr	Sulfur trioxide pyridine complex
t	Triplet (in NMR)
td	Triplets of doublets (in NMR)
tdd	Triplets of doublets of doublets (in NMR)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography

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Finally I wish to thank the most important person of all. My wonderful wife Joanne for her support, love and encouragement.

1.1: Introduction to peptidomimetics and proteases

Peptides and their analogues have long been used in medicinal chemistry as therapeutic agents against a range of pathological conditions. However, the presence of hydrolysable amide bonds means that peptide drugs possess the inherent disadvantages of a short half-life, poor oral bioavailability and poor pharmacokinetics. Considerable effort has been expended on finding peptidomimetics that possess the desired characteristics of the original peptides but , which are sufficiently "non-peptidic" to overcome the problems of metabolic degradation and poor bio-availability associated with peptides. One area where peptidomimetic research has been focussed for a number of decades, and is topic of this thesis, is the synthesis of protease inhibitors.

Proteases are globular, water soluble proteins that function as enzymes. The widely used term **protease** is synonymous with **peptidase,** but for simplicity the term protease will be used throughout this thesis. Proteases cleave or degrade other proteins by hydrolysing their component peptide bonds. This may appear an uninteresting reaction, but proteolytic enzymes are very important, accounting for approximately two per cent of genes in humans, infectious organisms, and other forms of life. Significantly, proteases regulate most physiological processes by controlling the activation, synthesis and turnover of proteins. They are consequently pivotal regulators of conception, birth, growth, maturation, ageing, diseases and death. However, it is mainly due to their importance in human health and disease that research into proteases has attracted a very significant amount of scientific interest for a number of years.

Proteases are categorised by the catalytic residue that effects enzymatic hydrolysis. Six classes of proteases are currently recognised[1];

- Serine proteases

- Threonine proteases
- Cysteine proteases
- Aspartic proteases
- Glutamic proteases
- Metallo-proteases

1.2: Protease inhibitors in the clinic

Proteases have been the subject of intense research for a number of years and a great number of these studies have proposed that their inhibitors could provide successful therapeutics for a wide range of diseases. Examples include, but are not limited to, cancer,[2-4] parasitic, fungal and viral infections; (e.g. schistosomiasis,[5, 6] malaria,[7, 8] HIV,[9-11] Hepatitis[12, 13] and Herpes),[14, 15] inflammatory,[16] respiratory,[17, 18] immunological,[19] cardiovascular[20] and neurodegenerative disorders.[21, 22]

The most compelling evidence of this is the number of compounds , which the pharmaceutical industry has either launched, or is currently conducting clinical studies upon (as of 2005).[23] Tables **1.1** to **1.5** list by protease classification the compounds , which (as of 2005) were known to be either in clinical trials or launched as a human drug.

Target	Indication	Drug Name	Company Name	Clinical Status
Caspase-1 (ICE)	Osteoarthritis, Psoriasis	VX-740-Pralnacasan	Vertex	Phase II
	Inflammatory disease	VX-765	Vertex	Phase II
Caspases-General	Hepatic dysfunction	IDN-6556	Idun	Phase IIb
	Myocardial Infarction, Cerebral Ischaemia	MX-1013	Maxim	Preclinical
Cathepsin K	Osteoporosis	AAE-581	Novartis	Phase II
	Osteoporosis	SB-462795	Glaxo Smith Kline	Phase I
Rhinovirus 3C	SARS	Ruprintrivir	Pfizer	Preclinical
	Common Cold	Rupintrivir		Phase II/III (Disc)

Table 1.1: Cysteine protease inhibitors in clinical development**.**

Target	Indication	Drug Name	Company Name	Clinical Status
Proteasome	Myeloma cancer	Bortezomib	Millenium	Launched
	Cerebral Ischaemia	MLN-519	Millenium	Phase I
	Anticancer	ER-807446	Eisai (Japan)	Pre-clinical
	Anticancer	TMC-95A		Pre-clinical

Table 1.2: Threonine protease inhibitors in clinical development**.**

Target	Indication	Drug Name	Company Name	Clinical Status
HIV-1 Protease	HIV/AIDS Infection	Ritonavir, Ritonavir soft gel	Abbott	Launched
	HIV/AIDS Infection	Lopinavir	Abbott	Launched
	HIV/AIDS Infection	Nelfinavir Mesylate	Pfizer	Launched
	HIV/AIDS Infection	Atazanavir Sulfate	Bristol-Myers Squibb	Launched
	HIV/AIDS Infection	Amprenavir	Vertex Pharmaceuticals	Launched
	HIV/AIDS Infection	Saquinavir, Saquinavir soft gel	Hoffmann-La Roche	Launched
	HIV/AIDS Infection	Crixivan	Merck & Co.	Launched
	HIV/AIDS Infection	Fosamprenavir Calcium	GlaxoSmith Kline	Launched
	HIV/AIDS Infection	Tipranavir	Pfizer	Phase III
	HIV/AIDS Infection	KNI-272	Japan Energy	Phase II (No Dev)
	HIV/AIDS Infection	TMC-114	Johnson & Johnson	Phase II
	HIV/AIDS Infection	NV-RX	Novartis	Phase II
	HIV/AIDS Infection	NAR-DG-35		Phase II (No Dev)
	HIV/AIDS Infection	VX-385	Vertex Pharmaceuticals	Phase I
	HIV/AIDS Infection	DPC-681		Phase I
	HIV/AIDS Infection	Ro-03-34649	Hoffmann-La Roche	Phase I
	HIV/AIDS Infection	PL-100	Procyon BioPharma	Preclinical
	HIV/AIDS Infection	C Sixty	C Sixty	Preclinical
	HIV/AIDS Infection	SM-309515	Sumitomo	Preclinical
	HIV/AIDS Infection	GS-9005	Gilead Sciences	Preclinical
	HIV/AIDS Infection	protease inibitor	Zapaq	Preclinical
Renin	Hypertension	Aliskiren	Novartis	Phase II (No Dev)
BACE	Alzheimer's Disease	LY-450139	Eli Lilly	Phase II
	Alzheimer's Disease	TGCN-001	The Genetics Company	Preclinical
	Alzheimer's Disease	β-secretase inhib	Acetilon	Preclinical
	Alzheimer's Disease	β-secretase inhib	Astex Technology	Preclinical
	Alzheimer's Disease	β-secretase inhib	Cellzome	Preclinical
	Alzheimer's Disease	β-secretase inhib	De Novo	Preclinical
	Alzheimer's Disease	β-secretase inhib	Elan	Preclinical
	Alzheimer's Disease	β-secretase inhib	Glaxo Smith Kline	Preclinical
	Alzheimer's Disease	β-secretase inhib	Locus	Preclinical
	Alzheimer's Disease	β-secretase inhib	NeoGenesis Pharm	Preclinical
	Alzheimer's Disease	β–secretase inhib	Sunesis	Preclinical
	Alzheimer's Disease	β–secretase inhib	Zapaq	Preclinical
	Alzheimer's Disease	TGC-2	The Genetics company	Preclinical

Table 1.3: Aspartic protease inhibitors in clinical development**.**

Target	Indication	Drug Name	Company Name	Clinical Status
ACE	Hypertension	Trandolapril, Enalapril, Captopril + 8 more		Launched
General Metalloprotease	Acne	Dermostat		Phase II
	Cancer, lung, non-small cell	Neovastat	Aeterna Zentaris	Phase III
	Cancer, Kaposi's sar	CMT-3	CollaGenex	Phase II
	Cancer, general	S-3304	Shionogi	Phase II
	Osteoarthritis	CPA-926	Kuhera Chemical	Phase II
	Cancer, general	XL-784	Exelixis	Phase I
	COPD	Ilomastat	Arriva	Preclincial
	Unspec. Indication	BMS-2 MMP inhibitors	Bristol-Myers Squibb	Preclincial
	Inflammation general	Metalloenzyme inhibitor	Serono	Preclincial
	Cancer, general	SC-77964	Pfizer	Preclincial
	Osteoarthritis	AZD-8955	Astra Zeneca	Preclincial
	Cancer, general	AMEP ligands	Bio Alliance Pharma	Preclincial
MMP-1	Peridontitis	Doxycycline	CollaGenex	Launched
	Inflammation, MS, Pancreatic Cancer	Marimastat	Vernalis	Phase III
	Cancer	Marimistat	British Biotech	Discontinued
MMP-2	Peridontal Disease	Periostat		Launched
	Cancer	Rebimistat		Phase III
	Prostate Cancer	Rebimastat	Celltech	Phase I
	Osteoarthritis	S-3536	Shionogi	Phase I
	Cancer, geneal	Ro-28-2653	Hoffmann-La Roche	Preclinical
MMP-3	Unspec. Indication	Neurolysin inhibitors	Aventis	Preclinical
MMP-8	Osteoarthritis	Glucosamine Sulfate	Rottapharm	Launched
MMP-9	Inflammation general	REGA-3G12	Biophage Pharma	Preclinical
	Prostate Cancer	MMP-9 inhibitor	AVI Biopharma	Preclinical
	Cancer, general	BDI-7800	Biopharmacopae	Preclinical
MMP-12	Multiple sclerosis	MMP-12 inhibitor	Serono	Preclinical
MMP-13	Osteoarthritis	MMP-13 inhibitor	Novartis	Preclinical
TACE	Arthritis, MS	BMS-561392	Bristol-Myers Squibb	Phase II
	Inflammation general	TMI-005	Wyeth	Phase I
	Asthma, COPD	PKF-241-266, 242-484	Novartis	Preclinical
NEP	Hypertension	Candroxatril	Pfizer	Phase III (disc)

Table 1.4: Metalloprotease inhibitors in clinical development**.**

Target	Indication	Drug Name	Company Name	Clinical Status
Thrombin	Venous Thrombosis	Ximelagatran	Astra Zeneca	Launched
	Thrombosis, general	Melagatran	Astra Zeneca	Pre-registration
	Arterial Thrombosis	Argatroban	Mitsubishi Pharma	Launched
	Venous Thrombosis	BIBR-1048	Boehringer Ingelheim	Phase III
	Thrombosis, general	MCC-977	Mitsubishi Pharma	Phase II
	Thrombosis, general	TGN-167, TGN-255	Trigen	Phase I
	Thrombosis, general	SSR-182289	Sanofi-Synthlabo	Phase I
	Thrombosis, general	AZD-0837	Astra Zeneca	Phase I
	Thrombosis, general	E-5555	Eisai	Phase I
	Venous Thrombosis	LB-30870	LG Life Sciences	Preclinical
Factor Xa	Thrombosis, Angina	DX-9065a	Daiichi	Phase II
	Venous thrombosis	DPC-906	BMS	Phase II
	Thrombosis	CI-1031	Berlex Biosciences	Phase II
	Venous thrombosis	JTV-803	Japan Tobacco	Phase II
NS3-protease	Hepatitis C Virus Infection	BILN-2061, Ciluprevir	Boehringer-Ingleheim	Phase II
	Hepatitis C Virus Infection	VX-950	Vertex	Phase I
Elastase	SIRS, Inflammation,	Sivelestat, Elaspol	Ono	Launched (Japan)
	COPD	Midesteine	Medea Research	Pre-registration (Italy)
	COPD	AE-3763	Dainippon	Pre-clinical
	COPD	R-448	Roche	Phase I
Broad-Spectrum	Pancreatitis, Inflammation	Nafamostat, FUT-175	Japan Tobacco	Launched
	Pancreatits	Camostat mesilate	Ono	Launched
Urokinase	Cancer, Gastrointestinal	WX-UK1	Wilex	Phase II
Chymase	Restenosis	NK-3201	Nippon Kayaku	Preclinical
DPP IV	Diabetes Type II	LAF-237	Novartis	Phase III
	Diabetes	MK-0431	Merck	PhaseII
	Diabetes	P32/98 (P3/01)	ProBiodrug	Phase I
	Diabetes	T-6666	Tanabe Seiyaku	Phase I
	Diabetes	NN-7201	Novo-Nordisk	Phase I

Table 1.5: Serine protease inhibitors in clinical development**.**

1.3: Importance of the β-strand in protease inhibitor design

With such compelling evidence for proteases being crucial mediators of mammalian physiology and disease, extensive research activity, particularly over the last 15 years, has resulted in a wealth of published information to assist in the development of new protease inhibitors.

A key concept , which underpins much of this protease inhibitor work is that conformation defines biological activity. This is not a new concept and in the area of peptidomimetic

research it has long been recognised that the biological function of a given peptide is achieved through the spatial arrangement of the peptide backbone. An excellent example of this is the three-dimensional 'bio-active' binding pattern displayed by the macrocyclic antibiotic vancomycin (**Figure 1.1)**.[24]

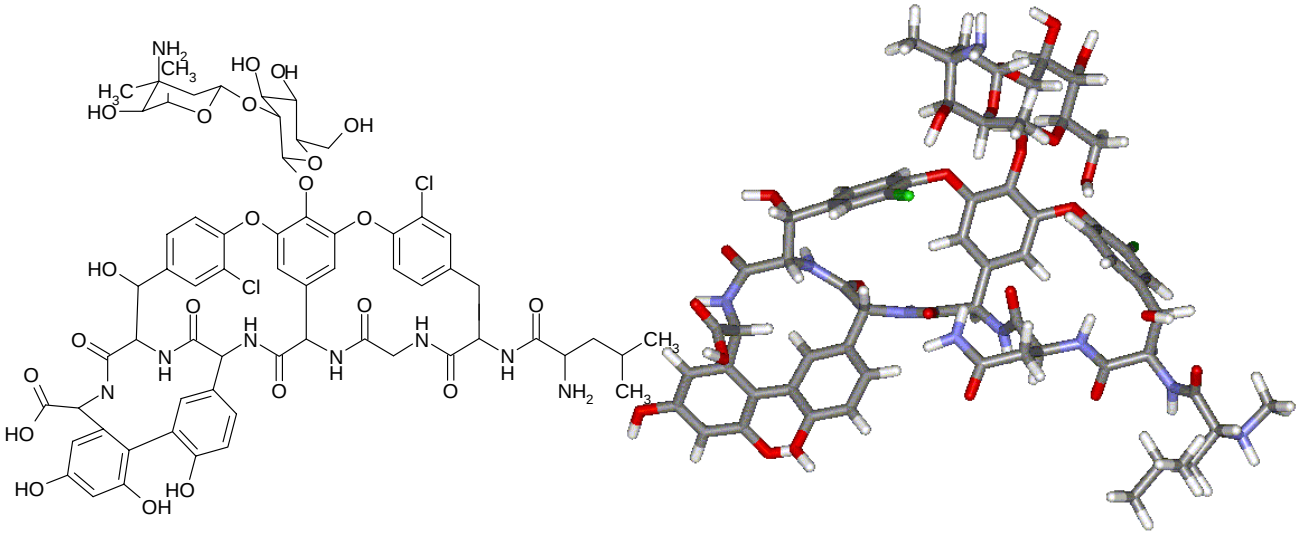
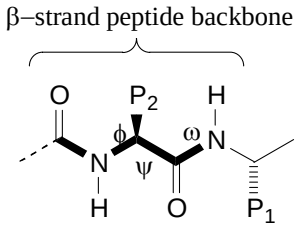


Figure 1.1: Chemical and crystal structure of Vancomycin.

As such a key concept in the design of peptidomimetics is if a "bioactive" conformation can be successfully stabilised then the resulting peptidomimetic is extremely likely to possess the corresponding biological activity. However, even with this knowledge, literature from the previous two decades suggests that *de-novo* peptidomimetic design for any given protease is extremely difficult. The primary reason for this is simply that often the "bioactive" conformation is not known.

This obstacle to protease inhibitor design is now much less of a problem. The reason for this is that during the late 1990s a breakthrough occurred in the understanding of the conformation responsible for the biological activity of protease inhibitors. As such it is now widely acknowledged that virtually all protease inhibitors bind to their target enzyme in an extended β-strand conformation. A β-strand motif is defined by the bond angles Φ, ψ, and ω, (with optimum angles of -120°, 120° and 180°, respectively **Figure 1.2**). In a peptide this affords a "zigzag" structure where the side chains are anti-parallel to one another **Figure 1.3**.



P_x = side chains binding into S_x recognition sites in the target protease

Figure 1.2: β-strand bond angles

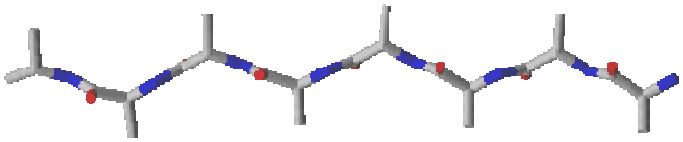


Figure 1.3: Zigzag structure of the β-strand

In 2005 a review[25] was published in , which over one thousand five hundred three dimensional crystal (X-ray) and solution (NMR) protease structures from the protein data bank were analysed. This analysis included substrates, products and inhibitors bound in the active sites of aspartic, serine, metallo, cysteine, and threonine proteases.

The results of this study provided unequivocal evidence that ligand binding in the active site of a protease occurs when the ligand adopts an extended β-strand conformation. Of the 1500 or so structures studied all but six bound the peptidic and non-peptidic ligands in this conformation. This is illustrated in **Figures 1.4 and 1.5. Figure 1.4** shows the overlay of the conformations of known inhibitors as bound to cathepsin K, a cysteine protease. **Figure 1.5** similarly shows the superimposition of known inhibitors of renin an aspartic protease. In both cases it is clearly evident that all the small molecules are in the β-strand conformation.

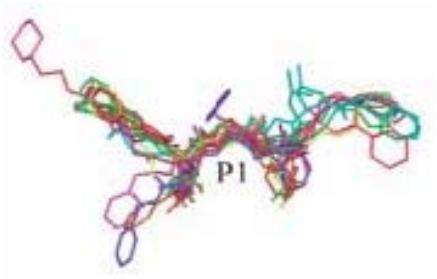


Figure 1.4: Overlay of known inhibitors bound in cathepsin K

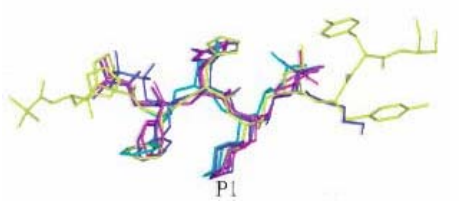


Figure 1.5: Overlay of known inhibitors bound in renin

The realisation that the β-strand motif is of fundamental importance for the binding of substrates and inhibitors to proteases is key to the work contained in this thesis. Traditionally inhibitor design has been specific for each protease, however with this knowledge it is proposed that it should be possible to formulate and design generic protease inhibitor templates. It is envisaged that synthesis of β-strand mimics, which act as templates, onto which additional recognition specific to each protease can be appended will provide a universal approach for the synthesis of protease inhibitors for all six classes of protease (**Figure 1.6**).

Figure 1.6: Schematic universal protease inhibitor design strategy

The fundamental principle underpinning this endeavour is that molecules which are pre-organised or "locked" into a β-strand conformation exhibit improved binding affinity, relative to their conformationally flexible analogues. This is due to a reduced loss of entropy upon binding.[26] This concept has been successfully demonstrated for an aspartic protease.[27]

1.4: Calpain as the prototype protease for the testing of β-strand mimics

In order to test the effectiveness of the β-strand mimics synthesised during this project the identification of a test protease was required. At the University of Canterbury the use of a calpain inhibition assay to determine the IC_{50} of a given calpain inhibitor was well established. The procedure for IC_{50} determination is described in **appendix 1**. This provided a convenient and reliable method to determine how good a particular β-strand mimic was. Furthermore cysteine proteases have not been well studied in terms binding β-strand motifs and as such this is important work towards achieving this goal. Moreover, as discussed in more detail later, the use of calpain as the test protease also provided opportunities for computational molecular modeling to be undertaken. However, most importantly, using

calpain as a test protease provided an opportunity to evaluate the best β-strand mimics *in-vivo* using the sheep cataract model established by our collaborators at the University of Lincoln.

The calpains are a family of calcium dependent cysteine proteases that are widely expressed in higher organisms. The most well characterised[28] calpains are the two ubiquitously expressed isoforms, calpain I (μ-calpain) and calpain II (m-calpain). The terms refer to the concentration of calcium required to induce their catalytic activity using *in-vitro* assays[29] (micromolar and millimolar respectively).

As shown in **Table 1.6** a number of pathologic conditions have been associated with disturbances of the calpain system. These will not be described in detail here as the primary objective of this thesis is the synthesis of β-strand mimics. It should however be noted that successful design, synthesis and conversion of a β-strand mimic into a calpain inhibitor provides potential opportunities to investigate the relationship between calpain over-activation and its associated pathologies. This point is considered in more detail in **Chapter 6** where we specifically target the development of cataract which results from the over activation of calpain.

Disease	Scientific rationale/observation
Cataract formation[30]	Ca^{2+} influx activates calpain II, the predominant calpain in the lens, cleaving α and β-crystallins. The crystallin fragments aggregate to form cataracts.
Myocardinal infarction[31-33]	Ca^{2+} homeostasis is lost in ischemic area, triggering inappropriate calpain activity. Protein and mRNA levels of calpain I and calpain II increase after myocardinal infarction
Neuronal ischemia (stroke)[34]	Calpastatin is degraded by calpain to a membrane-bound 50 kD polypeptide in ischemic brain tissue. Calpains participate in both apoptosis and necrosis which results in tissue damage in ischemic areas
Alzheimer's disease[35]	There is an increased amount of calpain II in the cytoslic but not the membranous fraction of the brain in the neurofibrillary tangles of the brain
Multiple Sclerosis[36, 37]	Levels of the 150 kD calpain specific degradation product of α-spectrin has been shown to increase in human multiple sclerosis plaques (by 50%). Degradation of a 68 kD neurofilament protein has been shown to be inhibited by a calpain inhibitor
Limb-girdle muscular dystrophy[38]	Associated with mutations in the gene encoding calpain III and probable loss of calpain III proteolytic activity
Obsessive compulsive disorder[39]	Erythrocytes from patients with OCD have significantly elevated calpain activities compared to normal controls.
Gastric cancer[40]	This is associated with a down regulation of calpain IX
Type 2 diabetes mellitus[41]	Mutations in calpain X are associated with an increased incidence of type 2 diabetes in some populations
Duchenne's and Becker's muscular dystrophies[42]	Associated with the absence or deficiency of dystrophin. This results in an increased Ca^{2+} level in muscle and inappropriate calpain activity

Table 1.6: Pathologic conditions associated with disturbances of the calpain system

References for Chapter 1

1. Hooper, N. M., Proteases: a primer. *Essays in Biochemistry* **2002,** 38, (Proteases in Biology and Medicine), 1-8.
2. Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M., Recent advances in matrix metalloproteinase inhibitor research. *Drug Discovery Today* **1996,** 1, (1), 16-26.
3. Johnson, L. L.; Dyer, R.; Hupe, D. J., Matrix metalloproteinases. *Current Opinion in Chemical Biology* **1998,** 2, (4), 466-471.
4. Yan, S. Q.; Sameni, M.; Sloane, B. F., Cathepsin B and human tumor progression. *Biological Chemistry* **1998,** 379, (2), 113-123.
5. Becker, M. M.; Harrop, S. A.; Dalton, J. P.; Kalinna, B. H.; McManus, D. P.; Brindley, P. J., Cloning and Characterization of the Schistosoma-Japonicum Aspartic Proteinase Involved in Hemoglobin Degradation. *Journal of Biological Chemistry* **1995,** 270, (41), 24496-24501.
6. Brindley, P. J.; Kalinna, B. H.; Dalton, J. P.; Day, S. R.; Wong, J. Y. M.; Smythe, M. L.; McManus, D. P., Proteolytic degradation of host hemoglobin by schistosomes. *Molecular and Biochemical Parasitology* **1997,** 89, (1), 1-9.
7. Silva, A. M.; Lee, A. Y.; Gulnik, S. V.; Majer, P.; Collins, J.; Bhat, T. N.; Collins, P. J.; Cachau, R. E.; Luker, K. E.; Gluzman, I. Y.; Francis, S. E.; Oksman, A.; Goldberg, D. E.; Erickson, J. W., Structure and inhibition of plasmepsin II, a hemoglobin-degrading enzyme from Plasmodium falciparum. *Proceedings of the National Academy of Sciences of the United States of America* **1996,** 93, (19), 10034-10039.
8. Li, Z.; Chen, X.; Davidson, E.; Zwang, O.; Mendis, C.; Ring, C. S.; Roush, W. R.; Fegley, G.; Li, R.; et al., Anti-malarial drug development using models of enzyme structure. *Chemistry & Biology* **1994,** 1, (1), 31-7.
9. Wlodawer, A.; Erickson, J. W., Structure-Based Inhibitors of Hiv-1 Protease. *Annual Review of Biochemistry* **1993,** 62, 543-585.
10. Darke, P. L.; Huff, J. R., HIV protease as an inhibitor target for the treatment of AIDS. *Advances in Pharmacology (San Diego, CA, United States)* **1994,** 25, 399-454.
11. West, M. L.; Fairlie, D. P., Targeting Hiv-1 Protease - a Test of Drug-Design Methodologies. *Trends in Pharmacological Sciences* **1995,** 16, (2), 67-75.
12. Kim, J. L.; Morgenstern, K. A.; Lin, C.; Fox, T.; Dwyer, M. D.; Landro, J. A.; Chambers, S. P.; Markland, W.; Lepre, C. A.; Omalley, E. T.; Harbeson, S. L.; Rice, C. M.; Murcko, M. A.; Caron, P. R.; Thomson, J. A., Crystal structure of the hepatitis C virus NS3

protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* **1996,** 87, (2), 343-355.

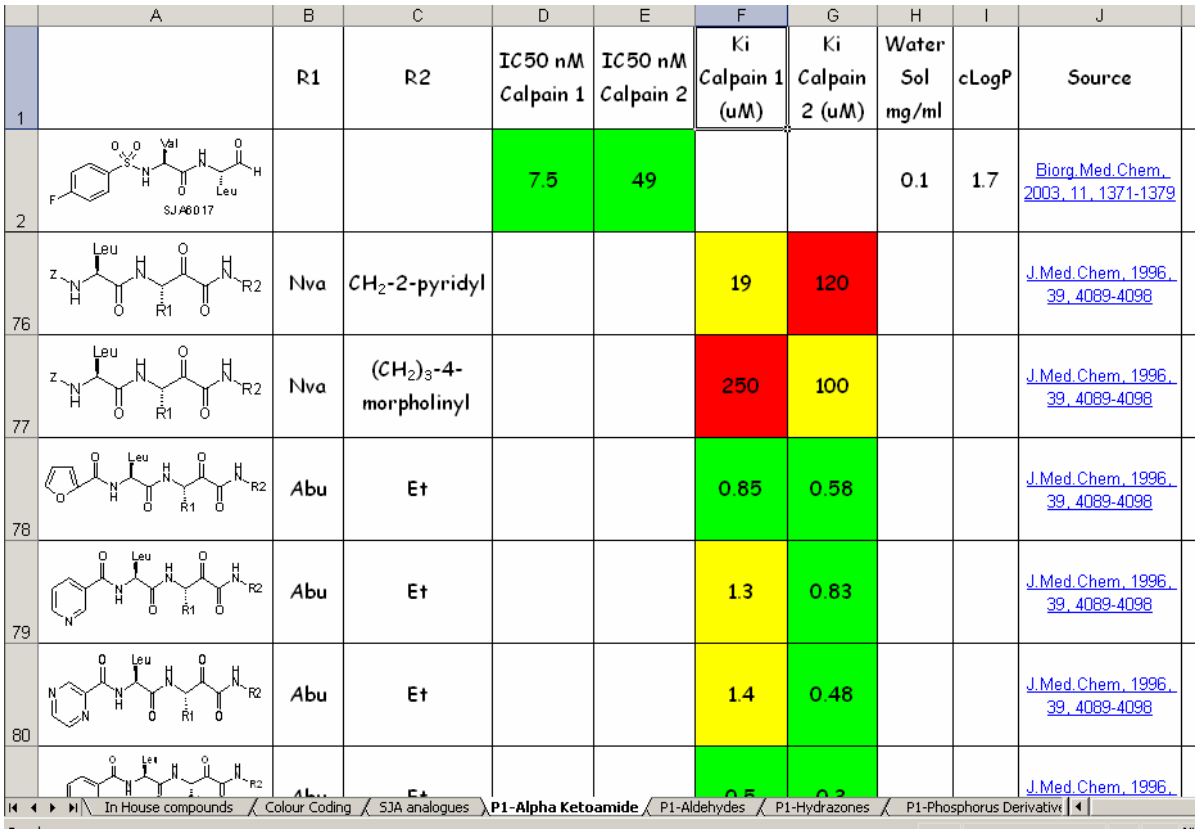
13. Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z., The crystal structure of hepatitis C virus NS3 proteinase reveals a trypsin-like fold and a structural zinc binding site. *Cell* **1996,** 87, (2), 331-342.
14. Gibson, W.; Hall, M. R., Assemblin, an essential herpesvirus proteinase. *Drug design and discovery* **1997,** 15, (1), 39-47.
15. Shieh, H. S.; Kurumbail, R. G.; Stevens, A. M.; Stegeman, R. A.; Sturman, E. J.; Pak, J. Y.; Wittwer, A. J.; Palmier, M. O.; Wiegand, R. C.; Holwerda, B. C.; Stallings, W. C., Three dimensional structure of human cytomegalovirus protease. *Nature* **1996,** 383, (6597), 279-282.
16. Bernstein, P. R.; Edwards, P. D.; Williams, J. C., Inhibitors of human leukocyte elastase. *Progress in Medicinal Chemistry* **1994,** 31, 59-120.
17. Fath, M. A.; Wu, X. J.; Hileman, R. E.; Linhardt, R. J.; Kashem, M. A.; Nelson, R. M.; Wright, C. D.; Abraham, W. M., Interaction of secretory leukocyte protease inhibitor with heparin inhibits proteases involved in asthma. *Journal of Biological Chemistry* **1998,** 273, (22), 13563-13569.
18. Tanaka, R. D.; Clark, J. M.; Warne, R. L.; Abraham, W. M.; Moore, W. R., Mast-Cell Tryptase - a New Target for Therapeutic Intervention in Asthma. *International Archives of Allergy and Immunology* **1995,** 107, (1-3), 408-409.
19. Hugli, T. E., Protease inhibitors: Novel therapeutic application and development. *Trends in Biotechnology* **1996,** 14, (11), 409-412.
20. Stubbs, M. T.; Bode, W., A Player of Many Parts - the Spotlight Falls on Thrombins Structure. *Thrombosis Research* **1993,** 69, (1), 1-58.
21. Selkoe, D. J., Neuroscience - Alzheimer's disease: Genotypes, phenotype, and treatments. *Science* **1997,** 275, (5300), 630-631.
22. Kim, T. W.; Pettingell, W. H.; Jung, Y. K.; Kovacs, D. M.; Tanzi, R. E., Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease. *Science* **1997,** 277, (5324), 373-376.
23. Abbenante, G.; Fairlie, D. P., Protease inhibitors in the clinic. *Medicinal Chemistry* **2005,** 1, (1), 71-104.
24. Nicolaou, K. C.; Boddy, C. N. C.; Brase, S.; Winssinger, N., Chemistry, biology, and medicine of the glycopeptide antibiotics. *Angewandte Chemie-International Edition* **1999,** 38, (15), 2097-2152.

25. Tyndall, J. D. A.; Nall, T.; Fairlie, D. P., Proteases universally recognize beta strands in their active sites. *Chemical Reviews* **2005,** 105, (3), 973-999.
26. Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L. L.; Burkett, B. A., Conformational selection of inhibitors and substrates by proteolytic enzymes: Implications for drug design and polypeptide processing. *Journal of Medicinal Chemistry* **2000,** 43, (7), 1271-1281.
27. Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P., Beta-Strand Mimetics. *Chemical Reviews* **2004,** 104, (12), 6085-6117.
28. Sorimachi, H.; Suzuki, K., The structure of calpain. *Journal of Biochemistry* **2001,** 129, (5), 653-664.
29. Perrin, B. J.; Huttenlocher, A., Calpain. *International Journal of Biochemistry & Cell Biology* **2002,** 34, (7), 722-725.
30. Shearer, T. R.; Ma, H.; Shih, M.; Fukiage, C.; Azuma, M., Calpains in the lens and cataractogenesis. *Methods in molecular biology (Clifton, N.J.)* **2000,** 144, 277-85.
31. Papp, Z.; van der Velden, J.; Stienen, G. J. M., Calpain-I induced alterations in the cytoskeletal structure and impaired mechanical properties of single myocytes of rat heart. *Cardiovascular Research* **2000,** 45, (4), 981-993.
32. Sandmann, S.; Yu, M. H.; Unger, T., Transcriptional and translational regulation of calpain in the rat heart after myocardial infarction - effects of AT(1) and AT(2) receptor antagonists and ACE inhibitor. *British Journal of Pharmacology* **2001,** 132, (3), 767-777.
33. Tsuji, T.; Ohga, Y.; Yoshikawa, Y.; Sakata, S.; Abe, T.; Tabayashi, N.; Kobayashi, S.; Kohzuki, H.; Yoshida, K. I.; Suga, H.; Kitamura, S.; Taniguchi, S.; Takaki, M., Rat cardiac contractile dysfunction induced by Ca2+ overload: possible link to the proteolysis of alpha-fodrin. *American Journal of Physiology-Heart and Circulatory Physiology* **2001,** 281, (3), H1286-H1294.
34. Blomgren, K.; Zhu, C. L.; Wang, X. Y.; Karlsson, J. O.; Leverin, A. L.; Bahr, B. A.; Mallard, C.; Hagberg, H., Synergistic activation of caspase-3 by m-calpain after neonatal hypoxia-ischemia - A mechanism of "pathological apoptosis"? *Journal of Biological Chemistry* **2001,** 276, (13), 10191-10198.
35. Tsuji, T.; Shimohama, S.; Kimura, J.; Shimizu, K., M-Calpain (calcium-activated neutral proteinase) in Alzheimer's disease brains. *Neuroscience Letters* **1998,** 248, (2), 109-112.

36. Shields, D. C.; Schaecher, K. E.; Saido, T. C.; Banik, N. L., A putative mechanism of demyelination in multiple sclerosis by a proteolytic enzyme, calpain. *Proceedings of the National Academy of Sciences of the United States of America* **1999,** 96, (20), 11486-11491.
37. Banik, N. L.; Matzelle, D.; Terry, E.; Hogan, E. L., A new mechanism of methylprednisolone and other corticosteroids action demonstrated in vitro: Inhibition of a proteinase (calpain) prevents myelin and cytoskeletal protein degradation. *Brain Research* **1997,** 748, (1-2), 205-210.
38. Ono, Y.; Shimada, H.; Sorimachi, H.; Richard, I.; Saido, T. C.; Beckmann, J. S.; Ishiura, S.; Suzuki, K., Functional defects of a muscle-specific calpain, p94, caused by mutations associated with limb-girdle muscular dystrophy type 2A. *Journal of Biological Chemistry* **1998,** 273, (27), 17073-17078.
39. Mundo, E.; Soldati, L.; Bellodi, L.; Bianchi, G., The calpain-calpastatin system in obsessive-compulsive disorder. *Biological Psychiatry* **1997,** 42, (3), 228-229.
40. Yoshikawa, Y.; Mukai, H.; Hino, F.; Asada, K.; Kato, I., Isolation of two novel genes, down-regulated in gastric cancer. *Japanese Journal of Cancer Research* **2000,** 91, (5), 459-463.
41. Horikawa, Y.; Oda, N.; Cox, N. J.; Li, X. Q.; Orho-Melander, M.; Hara, M.; Hinokio, Y.; Lindner, T. H.; Mashima, H.; Schwarz, P. E. H.; del Bosque-Plata, L.; Horikawa, Y.; Oda, Y.; Yoshiuchi, I.; Colilla, S.; Polonsky, K. S.; Wei, S.; Concannon, P.; Iwasaki, N.; Schulze, T.; Baier, L. J.; Bogardus, C.; Groop, L.; Boerwinkle, E.; Hanis, C. L.; Bell, G. I., Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nature Genetics* **2000,** 26, (2), 163-175.
42. Tidball, J. G.; Spencer, M. J., Calpains and muscular dystrophies. *International Journal of Biochemistry & Cell Biology* **2000,** 32, (1), 1-5.

2.1: Current calpain inhibitors in the scientific literature

The design and synthesis of calpain inhibitors is well documented in the literature[1]. An extensive review of both calpain inhibitors previously prepared at the University of Canterbury and those published in the literature was conducted. From this, a database capturing all the data in an easy to read and interpret format, with all data hyperlinked to source, was created. A section of this database is shown in **Figure 2.1**.



	R1	R2	IC50 nM Calpain 1	IC50 nM Calpain 2	Ki Calpain 1 (uM)	Ki Calpain 2 (uM)	Water Sol mg/ml	cLogP	Source
2			7.5	49			0.1	1.7	Biorg.Med.Chem. 2003, 11, 1371-1379
76	Nva	CH_2-2-pyridyl			19	120			J.Med.Chem, 1996, 39, 4089-4098
77	Nva	$(CH_2)_3$-4-morpholinyl			250	100			J.Med.Chem, 1996, 39, 4089-4098
78	Abu	Et			0.85	0.58			J.Med.Chem, 1996, 39, 4089-4098
79	Abu	Et			1.3	0.83			J.Med.Chem, 1996, 39, 4089-4098
80	Abu	Et			1.4	0.48			J.Med.Chem, 1996, 39, 4089-4098

In House compounds | Colour Coding | SJA analogues | P1-Alpha Ketoamide | P1-Aldehydes | P1-Hydrazones | P1-Phosphorus Derivativ

Figure 2.1: SAR database of calpain inhibitors

Qualitative analysis of this data revealed that virtually all calpain inhibitors are peptide based. This is because proteases bind their substrates in a groove or cleft. As shown in **Figure 2.2** amino acid side chains of substrates occupy enzyme sub-sites in the groove. These are designated,[2] with respect to the cleavable amide bond, as S3, S2, S1, S1', S2', S3'. The side chains bind to the corresponding substrate/inhibitor residues P3, P2, P1, P1', P2', P3'.

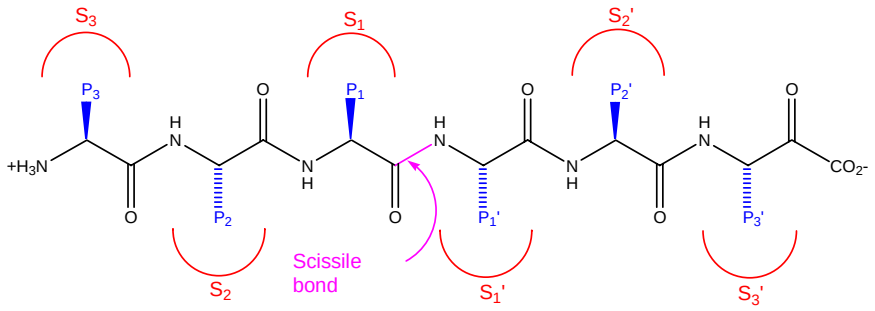


Figure 2.2: Substrate bound into a proteases

These peptide based calpain inhibitors can be divided into two distinct groups;

- Irreversible inhibitors
- Reversible inhibitors

Irreversible inhibitors

The irreversible calpain inhibitors are designed using well established mechanistic knowledge of cysteine proteases. As shown in **Figure 2.3** cysteine proteases hydrolyse amide bonds by first forming a thioacetyl intermediate which is then cleaved by an activated water molecule to regenerate the free thiol of the cysteine.

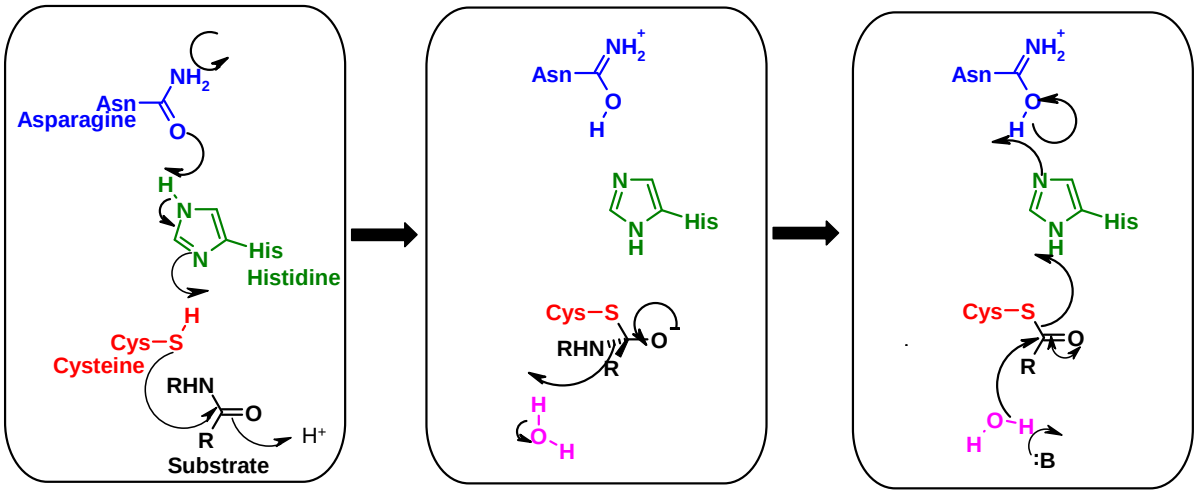


Figure 2.3: Mechanism of a cysteine protease

The mode of action of the irreversible inhibitors involves formation of a permanent covalent bond with the active site cysteine by virtue of the inhibitor containing a leaving group. Examples of these include peptide epoxide inhibitors (**2.1**), peptide aziridines (**2.2**), peptide

acyloxymethyl ketones (**2.3**), azapeptides (**2.4**), peptide benzotriazolooxy-methyl ketones (**2.5**) and peptide vinyl sulfones (**2.6**) (**Figure 2.4)**.

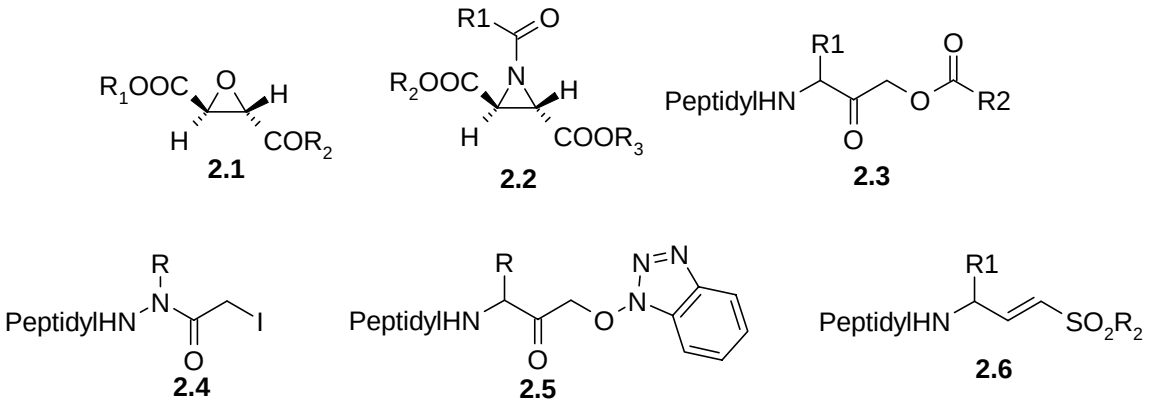


Figure 2.4: Various irreversible calpain inhibitors

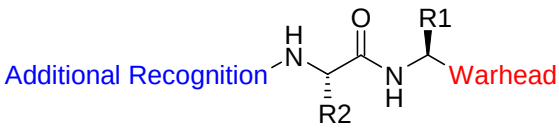
Reversible inhibitors

The vast majority of published reversible calpain inhibitors are based on generic structure **2.7**. This generalised structure consists of three segments;

- **An electrophilic warhead**. This reacts with the enzyme's active site cysteine to form a stable but reversible tetrahedral thiohemiacetal adduct. Examples of groups which are commonly used an electrophilic warhead include, aldehyde, α-dicarbonyl, α-diketone, α-ketoester, α-ketoamide and α-ketoacid.[1, 3-15] The most potent and commonly used electrophilic warhead is an aldehyde, although the use of α-ketoamide is also prevalent. The α-ketoamide analogues tend to be slightly less potent than their direct aldehyde equivalents.

- **A dipeptide backbone**. We have shown that this must be capable of binding to the enzyme in a β-strand conformation. This conformation ensures that the amino acid side chains (R1 and R2) are able to form the complementary (P1 and P2) interactions with the enzyme. This is not reported in the literature and our group is the first to investigate this using molecular modeling.

- **An additional recognition moiety**. This segment confers selectivity for calpain over other related enzymes. A wide variety of groups can be employed but typically a rather hydrophobic aromatic group is utilised.



2.7

As described above computational molecular modelling by Blair Stuart and Axel Neffe, within our group, demonstrated that the dipeptide backbone in these inhibitors adopts a β-strand conformation when bound to the enzyme active site. Inhibitors of the type **2.7** were shown to form three critical hydrogen bonds (Gly_{208} and Gly_{271}) to the enzyme (**Figure 2.5**). This was an important observation since it is in agreement with the known importance of the β-strand binding motif of other protease inhibitors.

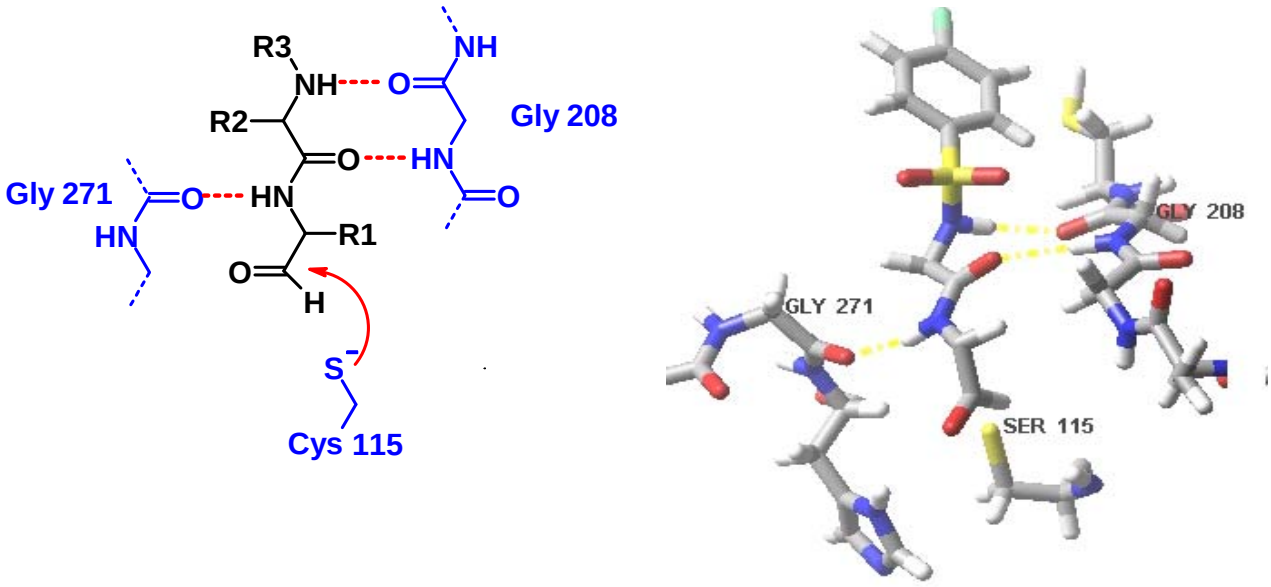


Figure 2.5: Binding of substrates/inhibitors in calpain

The work described in **Chapter 2** of this thesis focusses on the chemical synthesis of calpain inhibitors based around the generic structure **2.7,** with the objective of designing structures which adopt the requisite β-strand conformation.

2.2: Use of molecular modelling to design β-strand calpain inhibitors

An understanding of binding at the molecular level is required to accurately design β-strand mimics which possess a high binding affinity for calpain. The first crystal structures of calpain were published in 1999 and 2000.[16, 17] These reveal that calpain contains six domains. Each domain has a distinct function and the active site is located between domains I and II (**Figure 2.6**).

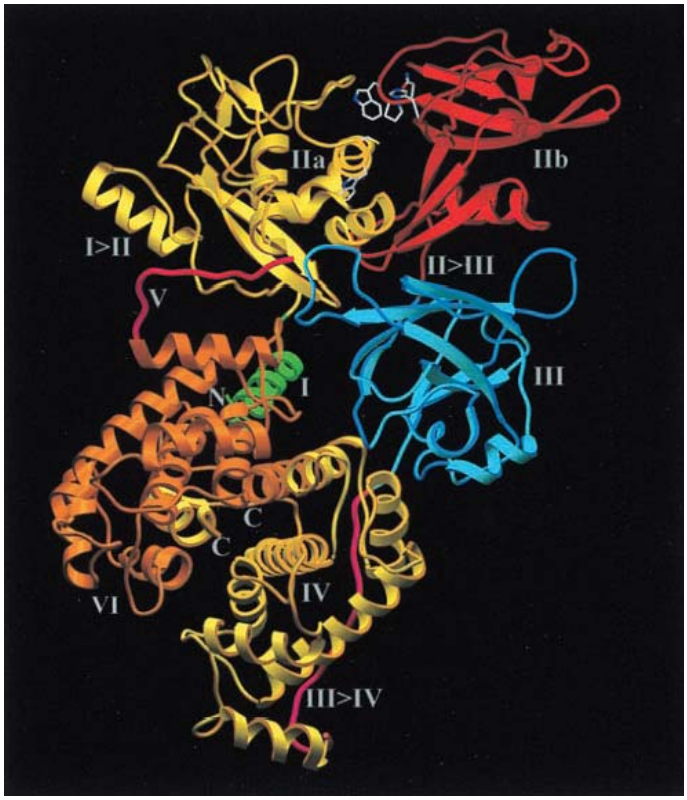


Figure 2.6: Calpain heterodimer.

However, these crystal structures do not provide sufficient structural information to allow careful scrutiny of the substrate binding cleft. The reason for this is that in these calcium free enzyme crystal structures the binding domains (domains I and II) are rotated and held slightly apart. This results in the active site catalytic triad residues not being in close proximity as is required for catalytic activity. As shown in **Figure 2.7,** the catalytic residues (cysteine and histidine) are separated in space by 10.5Å.

This problem was resolved when Moldoveanu *et al.* published, in 2002, a construct of calpain containing only the active site domains I-II. The structure of mini calpain I was published[18]

first, followed shortly afterwards by mini calpain II.[19] In both of these structures calcium ions were present and over 85% of the residues form essentially the same structure as domains I and II do in inactive calpain II.[19]

However, most importantly, as shown in **Figure 2.7**, the distance between the active site sulfur of cysteine 105 and the imidazole nitrogen of histidine 262 is now 3.7Å. This distance is the same as in the closely related cysteine protease papain. This observation, therefore, indicates that these mini-calpains contain the necessary structural detail for us to use molecular modeling to design new β-strand mimics as calpain inhibitors.

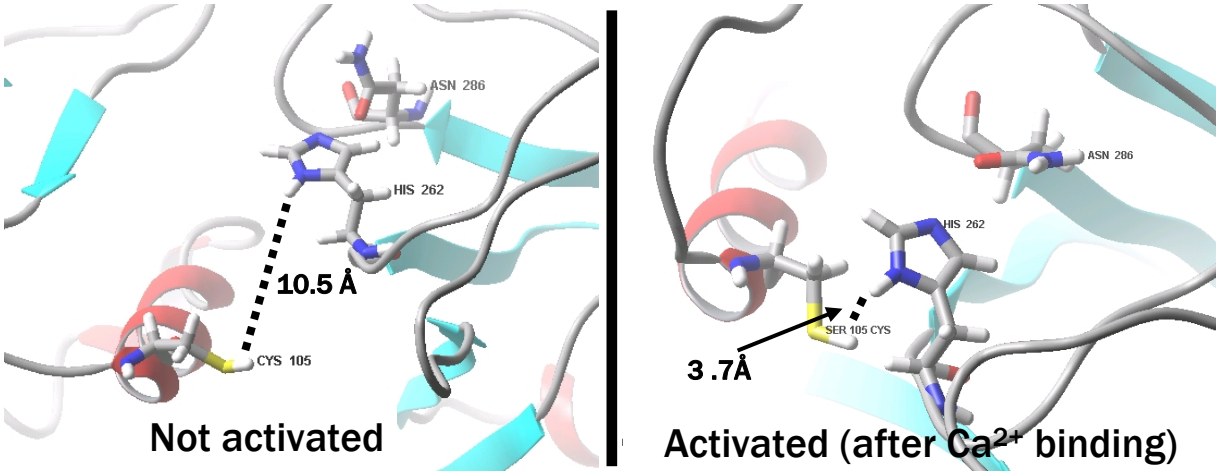


Figure 2.7: Calpain active site with and without Ca^{2+} binding

In addition to the publication of the crystal structures, enzyme inhibition assays using these mini-calpains were performed. These studies revealed that only mini calpain I had reasonable *in-vitro* catalytic activity. This was unexpected as the X-ray structure of the catalytic triad was very similar in both structures. The difference in enzymatic activity was rationalised by molecular modelling. In mini calpain II a tryptophan residue blocks the active site, whereas in mini calpain I this is not the case. As such mini calpain I was selected as the crystal structure for all molecular modeling studies reported in this thesis (**Figure 2.8**).

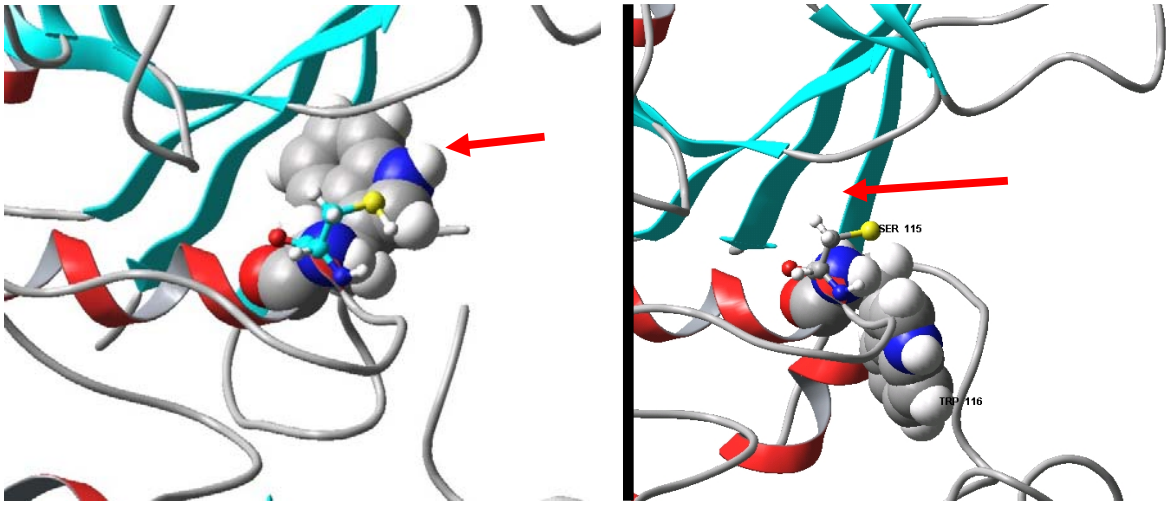


Figure 2.8: Comparison of the active sites of the mini calpains I and II

With this information in hand the mini calpain I structure (1KXR), obtained from the protein data bank (pdb), was prepared for docking studies. This required a number of steps to be performed:

- water molecules and ions were deleted;
- hydrogens atoms were added;
- The active site Ser_{115} was mutated to Cys_{115};
- Cys_{115} was deprotonated and His_{272} protonated to most closely match physicological conditions;
- The structure was minimised using the OPLS 2001 force field with a generalised Born/surface area (GB/SA) water model over 500 iterations. All residues within a 5 Å distance of the calcium ions of the crystal structure were kept "frozen" during this minimization. The RMSD of the minimised structure to the crystal structure was 0.96 Å for the heavy atoms (C, N, O, S);
- The docking grid was defined as the centroid of residues Cys_{115}, Gly_{208}, and Gly_{271}. This was generated with GLIDE 3.5 using the default settings. The centre of any docked ligand had to be within a 12 Å box.

The resulting structure was used in all molecular modelling experiments used in this thesis. The *in-silico* design of drugs, and protease inhibitors in general, using molecular modelling is well documented throughout the literature.[20] Numerous commercial programs are available for this task. However at the University of Canterbury all molecular was conducted using the Schrödinger suite 2005. GLIDE[21] was used as the docking program using the following parameters:

- OPLS2001 force field,
- Standard precision mode,
- 90000 poses per ligand,
- A scoring window of 5000 for keeping initial poses,
- 10000 poses per ligand for energy minimisation,
- Energy minimisation with a distance dependent dielectric constant of 2 and a maximum of 5000 conjugate gradient steps,
- 10 poses per ligand were saved for evaluation.

A number of parameters were obtained from this docking program. The three most important parameters for the design of a β-strand mimic as a calpain inhibitor are:

- **Does the molecule bind as a β-strand mimic**? Visual and mathematical (see **Section 4.1**) examination of the molecule bound into the active site reveals whether or not it binds in a β-strand conformation;

- **Glide Score.** This numerical score is a reflection of the relative binding energy of each ligand to the enzyme. A large number of parameters are used to calculate this score, however the number and strength of hydrogen bonds that the molecule forms with the enzyme active site is the largest of these components;

- **Warhead to active site Cys_{105} distance.** Glide calculates the distance between the carbon of the electrophilic warhead group and Cys_{105}. Modelling known calpain inhibitors determined that the warhead to Cys_{105} distance was less than 5Å for all potent inhibitors. All molecules which bind with a greater distance than this were poor inhibitors. As such a cut-off of 5Å was used to determine if a modelled compound could potentially be a potent calpain inhibitor.

Ligands with good initial docking scores were then subjected to re-docking using the extra precision docking mode in GLIDE. The XP docking protocol provided the most accurate results but as this was a much slower docking protocol than the standard precision mode it was only used after SP docking had generated a list of promising compounds. The XP docking was used to filter out "false positives" obtained from the much quicker standard precision docking. The same docking parameters described above were used and same three scoring parameters were obtained.

2.3: Patentability evaluation

As the synthesis of the new β-strand mimics was based on the well studied generic structure **2.7** an exhaustive patent search was undertaken to ensure that compounds synthesised in this thesis were patentable**.**

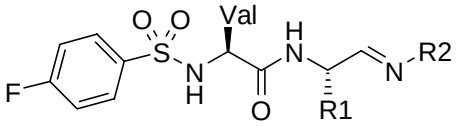
A total of fifty four calpain inhibitor patents were obtained from a Scifinder search performed in early March 2004. None of these considered the role of β-strand conformation in binding. As the objective of the Abell research group was to patent any new β-strand mimic calpain inhibitor as an anti-cataract agent four key patents were identified which were based on generic structure **2.7** and claimed the use of the compounds contained within as anti-cataract agents. All four were published by Senju pharmaceuticals, Japan.

Patent 1. Published 9.10.1999[22]

2.8

This patents the most potent inhibitors prepared by Senju. R^1 covers a very wide variety of moieties. R^2 and R^3 are defined as both the R group of an (L)-amino acid and as a three to seven membered carbocycle. R^4 is the R group of an (L)-amino acid. The warhead is defined as an aldehyde. The extensive definitions of R^1 essentially make it very difficult, if not impossible, to be outside the scope of IP coverage with an N-sulfonamide dipeptide aldehyde based inhibitor. The only feasible way to be outside patent coverage would be to use an alternative warhead. This motivated us to cease work on compounds of this type.

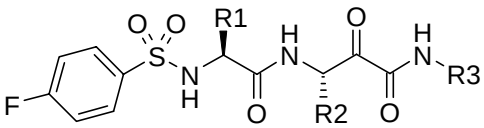
- **Patent 2 – Published 6.12.2001**[23]



2.9

This has limited coverage compared to patent 1. It is potentially possible to break this patent by simply using alternative N-substituents. However, inhibitors incorporating a hydrazone as a warhead are known to be poor inhibitors. As such it was decided that no work was to be performed on these types of inhibitors.

- **Patent 3 – Published 29.1.2004**[24]

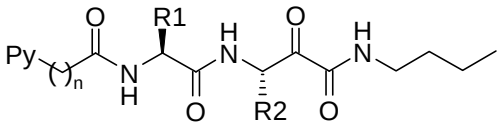


2.10

This covers a very wide range of alpha-ketoamides. It defines a single group as the N-substituent. Therefore, as for patent 2, using alternative N-substituents may be sufficient to obtain a novel patentable inhibitor. However, alpha-ketoamide synthesis is not trival and

generally alpha-ketoamides are less potent inhibitors that their coresponding aldehyde analogues. Nevertheless these still represent synthetic targets of interest.

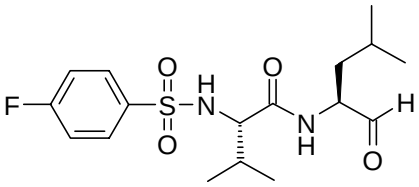
- **Patent 4 – Published 12.2.2004**[25]



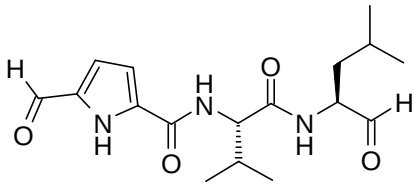
2.11

This patent covers a handful of compounds based on a defined alpha-ketoamide. The pyridyl moieties are attached by varying tether chain lengths on the N-terminus. These compounds were designed to increase aqueous solubility. Again this provides opportunities, especially considering that the pyridyl moieties are attached as an amide rather than as a sulfonamide. As such, a mix and match strategy between this patent and the first could be expected to provide potent and patentable inhibitors.

In addition to these options a previous member of the Abell group had synthesised dialdehyde **2.13**. This had been determined to have an IC_{50} of 30 nM, which was more potent than the best published inhibitor of type **2.7**, SJA-6017 (**2.12**) Furthermore molecular modelling by Blair Stewart indicated that this was an excellent β-strand mimic and the fact that it was not covered by the scope of any published patent made this a very attractive starting point for the synthesis of acyclic β-strand mimics.



2.12 (SJA-6017)

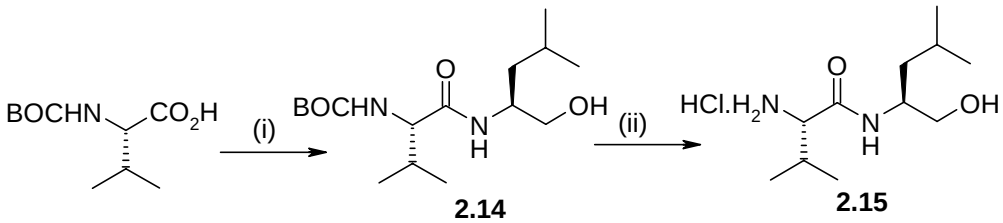


2.13

As such **2.13** was used as a template to design and model a number of close analogues. From this eleven were selected for synthesis and IC_{50} determination **(Table 2.1).** Of these eight compounds were prepared in this thesis (**Schemes 2.2** and **2.3)**.

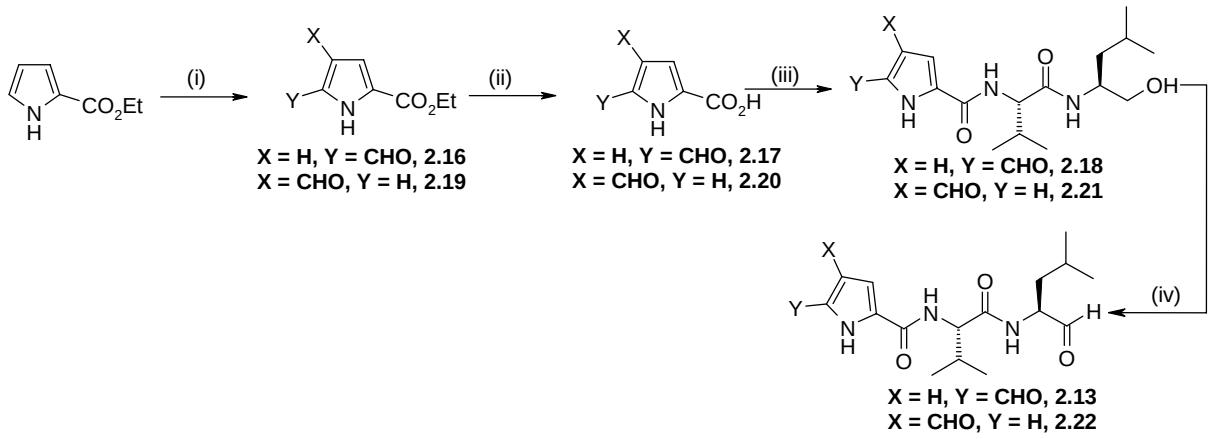
2.4: Design, synthesis and testing of N-heterocyclic dipeptide aldehyde calpain inhibitors

To minimise the number of synthetic steps required a synthetic strategy that allowed preparation of a range of related structures using a common intermediate was devised. As shown in **Scheme 2.1** the amino alcohol **2.14** provided such a key intermediate. Commercial *N*-BOC-(L)-Val-H was coupled with (L)-leucinol using standard HATU peptide coupling conditions to yield *N*-BOC-Val-Leu-OH (**2.15**). The use of HATU as a peptide coupling reagent ensures that the stereochemical integrity of the coupling partners is maintained. This is widely acknowledged in the literature. Cleavage of the BOC protecting group was achieved using 4M hydrogen chloride in 1,4-dioxane.



Scheme 2.1. *Reagents and Conditions:* **(i)** HATU, DIPEA, (L)-leucinol, DMF, (68%); **(ii)** 4M HCl in 1,4-dioxane, (100%)

The 5-and 4-formyl pyrrole analogues were both prepared from commercial pyrrole-2-ethyl ester using the procedure published by Martyn and *et al.*[26] (**Scheme 2.2**). Formylation of pyrrole-2-ethyl ester afforded a mixture of 4-(**2.19**) and 5-(**2.16**) formylated pyrrole-2-ethyl esters in a ratio of 1:1.9. Hydrolysis of these under basic conditions and coupling of the resultant acids with dipeptide alcohol **2.15** gave the required alcohols **2.18** and **2.21**. These were oxidised with sulfur trioxide pyridine complex in DMSO/DCM under basic conditions to give the desired aldehydes **2.13** and **2.22**.

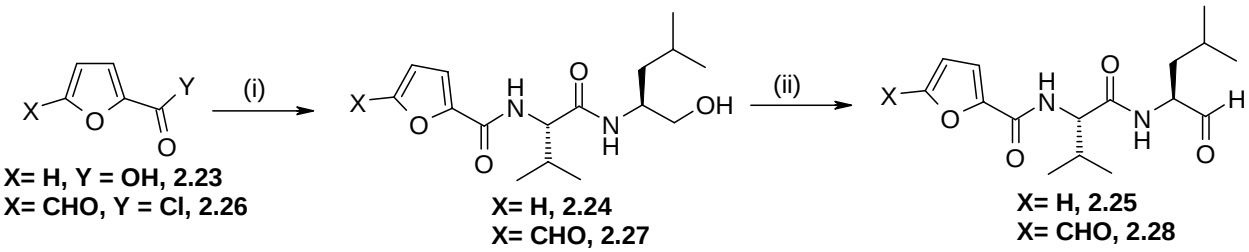


Scheme 2.2. *Reagents and Conditions:* **(i)** $POCl_3$, DMF, DCE, **X = H, Y = CHO** (52%) and **X = CHO, Y = H** (27%)

For X = H, Y = CHO; (ii) KOH, H_2O, (96%); **(iii)** EDC, HOBt.H_2O, DIPEA, **2.15,** DCM (39%); **(iv)** SO_3.Pyr, DIPEA, DMSO, DCM, (66%)

For CHO, Y = H; (ii) KOH, H_2O, (94%); **(iii)** EDC, HOBt.H_2O, DIPEA, **2.15,** DCM (24%); **(iv)** SO_3.Pyr, DIPEA, DMSO, DCM, (18%)

The 2-furyl and 2-(5-formyl)-furyl analogues were prepared as shown in **Scheme 2.3.** Coupling of 5-formyl-acid chloride **2.26** or 5-unsubstitued carboxylic acid **2.23** to amine **2.15** afforded dipeptide alcohols **2.24** and **2.27** respectively. Oxidation of each of these with sulfur trioxide pyridine complex in DMSO/DCM, under basic conditions, gave aldehydes **2.25** and **2.28**.

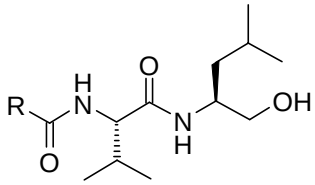


Scheme 2.3. *Reagents and Conditions:* **For X = H, Y = Cl;** (i) **2.15**, DMAP, Et_3N, DCM, (28%); (ii) SO_3.Pyr, DIPEA, DMSO, DCM, (90%)

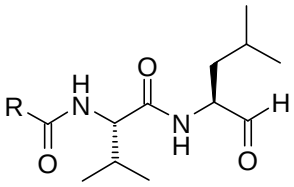
For X = CHO, Y = H; (i) EDC, HOBt.H_2O, DIPEA, **2.15,** DCM (24%); (iv) SO_3.Pyr, DIPEA, DMSO, DCM, (56%)

Summary of assay results

Compounds **2.13**, **2.18** and **2.21 – 2.32** were then assayed against calpain II and the results are presented in **Table 2.1**.



Type A



Type B

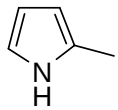
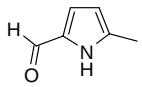
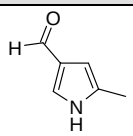
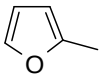
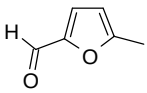
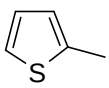
R substituent	Type	Compound Number	Glide Score	Are the 3 β-strand H-bonds present	Warhead distance to Cys (Å)	IC_{50} (nM)
	A	**2.29 (M Jones)**	-6.60	Yes	3.94	15800
	B	**2.30 (M Jones)**	-3.30	Yes	3.50	140
	A	**2.18**	-6.90	Yes	3.55	970
	B	**2.13**	-7.20	Yes	3.70	30
	A	**2.21**	-5.10	No	4.30	>250000
	B	**2.22**	-5.90	No	>5	>250000
	A	**2.24**	-6.00	Yes	3.40	176400
	B	**2.25**	-6.50	Yes	3.70	130
	A	**2.27**	-5.90	No	3.47	119900
	B	**2.28**	-3.40	No	6.82	1440
	A	**2.31 (S Mcnabb)**	-5.70	Yes	3.68	174000
	B	**2.32 (S Mcnabb)**	-5.20	Yes	3.62	730

Table 2.1: SAR of N-heterocyclic-Val-Leu-CHO calpain inhibitors

From **Table 2.1** three SAR implications can be inferred;

- It is vital that the inhibitors are able to adopt the β-strand conformation (**Figure 2.9**). In **Table 2.1** the compounds (**2.21, 2.22, 2.27** and **2.28**) which are shown by molecular modelling to be unable to form the β-strand conformation are accordingly poor to very poor calpain inhibitors.

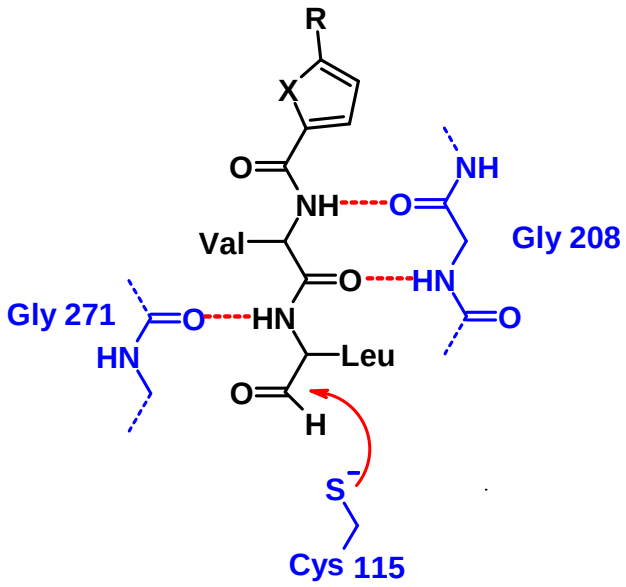


Figure 2.9: β-strand binding of N-heteroaromatic dipeptide aldehyde calpain inhibitors

- A 5-formyl substituent on the heterocycle can either increase or decrease calpain inhibition. In the case of the furyl derivates (compare **2.25** and **2.28**) this substitution results in a ten fold decrease in protease inhibition. This is possibly due to repulsion between the carbonyl of the formyl group and the oxygen of the heterocycle. This results in the molecule not being able to adopt a low energy β-strand conformation and hence decreased calpain inhibition is observed. In the case of the pyrrole derivatives (compare **2.13** and **2.30**) a 3.5 fold increase in protease inhibition is obtained on formyl substitution. This is predicted by the modelling scores in **Table 2.1** and is a result of the 5-formyl-pyrrole group binding in novel mode (**Figure 2.10**).

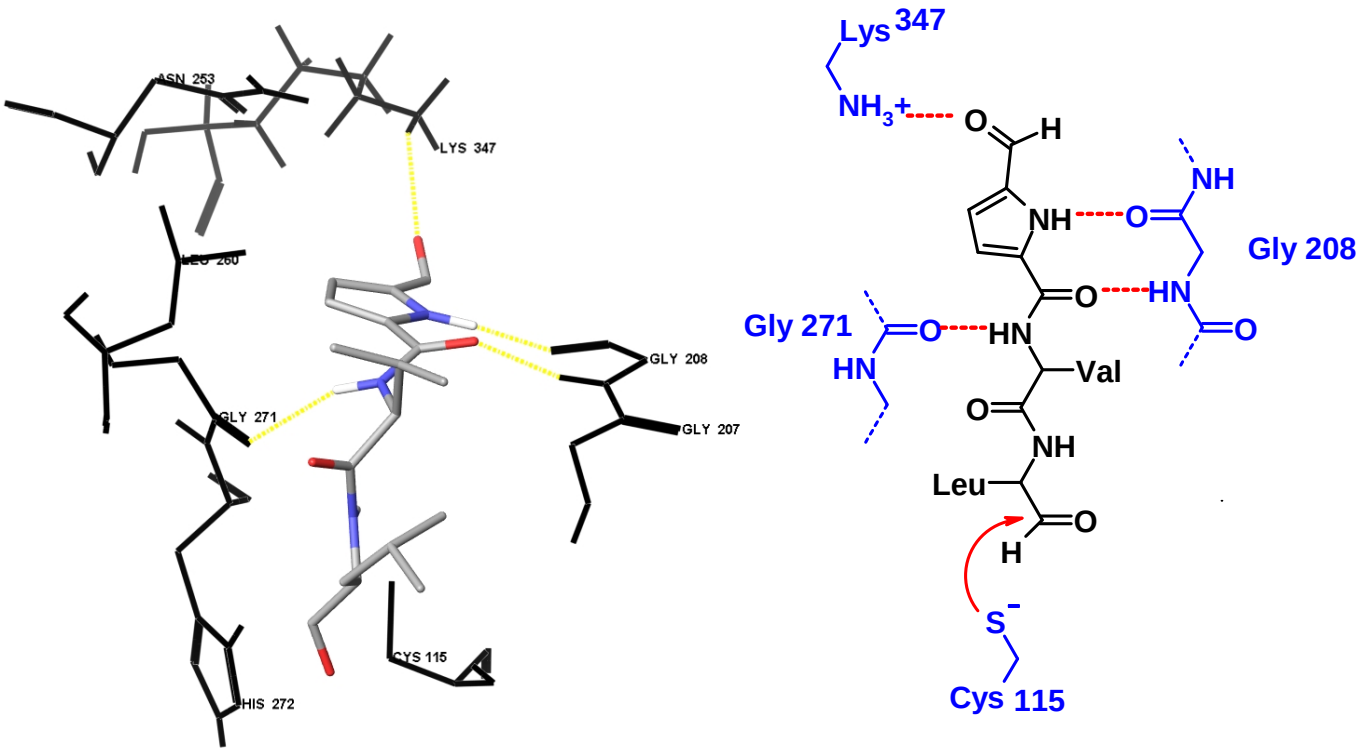


Figure 2.10: Novel binding mode of **2.13**

In this novel binding mode the carbonyl of the formyl group, the pyrrole NH and the carbonyl of the amide all contribute β-strand forming hydrogen bonds. These atoms have replaced or 'mimic' the hydrogen bonds formed by the carbonyl and the two NHs in a usual dipeptide backbone β-strand conformation. Moreover, in **2.13** the hydrogen bonding atoms are "locked" or conformationally constrained into a β-strand. As such **2.13** has a lower entropy loss upon binding compared to other the "acyclic" β-strands mimics in **Table 2.1**. This is an extremely important observation and throughout the reminder of this thesis the strategy of attempting to conformationally constrain compounds into the 'bioactive' β-strand conformation is a recurring theme.

2.5: Conclusions and future work

The most important conclusion derived from **Chapter 2** is that all the potent inhibitors are able to adopt a β-strand. A modelling protocol using a calpain construct of domains I and II has been developed so that the SAR of current calpain inhibitors can be rationalised. This

protocol was then applied to the design of a series of acyclic N-heteroaromatic dipeptide aldehydes. These new β-strand mimics were outside the scope of any current patents and the most potent of these was **2.13** with an IC_{50} against calpain II of 30 nM.

The possibility to undertake further work in this area is limited. Investigation of the novel binding mode of **2.13** is worthwhile to confirm that a new and important non-peptidic β-strand binding motif has been identified. However apart from this section of work the area is extremely competitive and well patented. Macrocyclisation as described in **Chapter 4** is a much more worthwhile undertaking if the objective is to synthesise novel patentable β-strand mimics.

References for Chapter 2

1. Neffe, A. T.; Abell, A. D., Developments in the design and synthesis of calpain inhibitors. *Current Opinion in Drug Discovery & Development* **2005,** 8, (6), 684-700.

2. Schechte.I; Berger, A., On Size of Active Site in Proteases .I. Papain. *Biochemical and Biophysical Research Communications* **1967,** 27, (2), 157-&.

3. Wells, G. J.; Bihovsky, R., Calpain inhibitors as potential treatment for stroke and other neurodegenerative diseases: recent trends and developments. *Expert Opinion on Therapeutic Patents* **1998,** 8, (12), 1707-1727.

4. Tao, M.; Bihovsky, R.; Wells, G. J.; Mallamo, J. P., Novel peptidyl phosphorus derivatives as inhibitors of human calpain I. *Journal of Medicinal Chemistry* **1998,** 41, (20), 3912-3916.

5. Nakamura, M.; Yamaguchi, M.; Sakai, O.; Inoue, J., Exploration of cornea permeable calpain inhibitors as anticataract agents. *Bioorganic & Medicinal Chemistry* **2003,** 11, (7), 1371-1379.

6. Nakamura, M.; Inoue, J., Exploration of peptidyl hydrazones as water-soluble calpain inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2002,** 12, (12), 1603-1606.

7. Lubisch, W.; Moller, A., Discovery of phenyl alanine derived ketoamides carrying benzoyl residues as novel calpain inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2002,** 12, (10), 1335-1338.

8. Lubisch, W.; Hofmann, H. P.; Treiber, H. J.; Moller, A., Synthesis and biological evaluation of novel piperidine carboxamide derived calpain inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2000,** 10, (19), 2187-2191.

9. Li, Z. Z.; OrtegaVilain, A. C.; Patil, G. S.; Chu, D. L.; Foreman, J. E.; Eveleth, D. D.; Powers, J. C., Novel peptidyl alpha-keto amide inhibitors of calpains and other cysteine proteases. *Journal of Medicinal Chemistry* **1996,** 39, (20), 4089-4098.

10. Inoue, J.; Nakamura, M.; Cui, Y. S.; Sakai, Y.; Sakai, O.; Hill, J. R.; Wang, K. K. W.; Yuen, P. W., Structure-activity relationship study and drug profile of N-(4-fluorophenylsulfonyl)-L-valyl-L-leucinal (SJA6017) as a potent calpain inhibitor. *Journal of Medicinal Chemistry* **2003,** 46, (5), 868-871.

11. Donkor, I. O.; Zheng, X. Z.; Han, J.; Lacy, C.; Miller, D. D., Significance of hydrogen bonding at the S-1 ' subsite of calpain I. *Bioorganic & Medicinal Chemistry Letters* **2001,** 11, (13), 1753-1755.

12. Donkor, I. O.; Han, J.; Zheng, X. Z., Design, synthesis, molecular modeling studies, and calpain inhibitory activity of novel alpha-ketoamides incorporating polar residues at the P-1'-position. *Journal of Medicinal Chemistry* **2004,** 47, (1), 72-79.

13. Donkor, I. O., A survey of calpain inhibitors. *Current Medicinal Chemistry* **2000,** 7, (12), 1171-1188.

14. Chatterjee, S.; Senadhi, S.; BozyczkoCoyne, D.; Siman, R.; Mallamo, J. P., Nonpeptidic inhibitors of recombinant human calpain I. *Bioorganic & Medicinal Chemistry Letters* **1997,** 7, (3), 287-290.

15. Bihovsky, R.; Tao, M.; Mallamo, J. P.; Wells, G. J., 1,2-Benzothiazine 1,1-dioxide alpha-ketoamide analogues as potent calpain I inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2004,** 14, (4), 1035-1038.

16. Hosfield, C. M.; Elce, J. S.; Davies, P. L.; Jia, Z. C., Crystal structure of calpain reveals the structural basis for Ca2+-dependent protease activity and a novel mode of enzyme activation. *Embo Journal* **1999,** 18, (24), 6880-6889.

17. Strobl, S.; Fernandez-Catalan, C.; Braun, M.; Huber, R.; Masumoto, H.; Nakagawa, K.; Irie, A.; Sorimachi, H.; Bourenkow, G.; Bartunik, H.; Suzuki, K.; Bode, W., The crystal structure of calcium-free human m-calpain suggests an electrostatic switch mechanism for activation by calcium. *Proceedings of the National Academy of Sciences of the United States of America* **2000,** 97, (2), 588-592.

18. Moldoveanu, T.; Hosfield, C. M.; Lim, D.; Elce, J. S.; Jia, Z.; Davies, P. L., A Ca2+ switch aligns the active site of calpain. *Cell (Cambridge, MA, United States)* **2002,** 108, (5), 649-660.

19. Moldoveanu, T.; Hosfield, C. M.; Lim, D.; Jia, Z. C.; Davies, P. L., Calpain silencing by a reversible intrinsic mechanism. *Nature Structural Biology* **2003,** 10, (5), 371-378.

20. Tyndall, J. D. A.; Nall, T.; Fairlie, D. P., Proteases universally recognize beta strands in their active sites. *Chemical Reviews* **2005,** 105, (3), 973-999.

21. Krovat, E. M.; Steindl, T.; Langer, T., Recent Advances in Docking and Scoring *Current Computer-Aided Drug Design* **2005,** 1, 93-102.

22. Azuma, M.; Yoshida, Y.; Sakamoto, Y.; Inoue, J. Pharmaceutical composition containing an arylsulfonyl dipeptide aldehyde for prophylaxis and therapy of diseases associated with ocular fundus tissue cytopathy. 99-JP1031
9944624, 19990303., 1999.

23. Nakamura, M.; Inoue, J. Preparation of N-(4-fluorobenzenesulfonyl)dipeptide aldehyde hydrazone derivatives as cysteine protease inhibitors and use thereof in medicines. 2001-JP10667
2002048096, 20011206., 2002.

24. Nakamura, M.; Inoue, J. Preparation of a-ketoamide derivatives as calpain inhibitors. 2003-JP8878
2004009537, 20030711., 2004.

25. Nakamura, M.; Inoue, A. Calpain-inhibiting optically active pyridine derivatives and pharmaceuticals containing them. 2002-200403
2004043330, 20020709., 2004.

26. Martyn, D. C.; Vernall, A. J.; Clark, B. M.; Abell, A. D., Ring-deactivated hydroxyalkylpyrrole-based inhibitors of alpha-chymotrypsin: synthesis and mechanism of action. *Organic & Biomolecular Chemistry* **2003,** 1, (12), 2103-2110.

3.1: Conformational constraint of peptidomimetics

Nature has found many intriguing and elegant ways to reduce the flexibility of peptides and proteins in order to increase both affinity and selectivity for an interacting partner molecule. Reduction of conformational flexibility by hydrogen bonding and formation of disulfide bridges are the two most well known ways to control shape.[1] More sophisticated modes for reducing flexibility include the formation of sulfide bridges, zinc fingers, and multiple side chain knotting.[2]

The principle of reducing flexibility and thereby limiting the unfavorable entropy loss upon binding is a widely applied principle in peptidomimetic design and drug discovery.[3] The primary reason for this is because the correct choice of conformational constraint often confers both improved biological activity and enzyme/receptor selectivity. This undertaking is usually quite a challenge as often the biologically active conformation of a peptide is not known. However, as described in **Section 1.3** it is now known that proteases almost universally bind their substrates in an extended β-strand conformation.

As shown in **Chapter 2**, for the first time, molecules that adopt a β-strand conformation are generally excellent calpain inhibitors. As such the next logical step in the design process was to prepare protease inhibitors which were "locked" by conformational constraint into the 'bio-active' β-strand conformation. We proposed to use ring closing metathesis (RCM) to synthesise a range of cyclic conformationally constrained β-strand templates.

As shown in **Figure 3.1** it was proposed that appendage of appropriate address regions to a β-strand template, for a particular protease, would confer specificity for that protease. From analysis of the vast literature published on protease inhibitor design it is possible to identify

numerous groups which would potentially validate this generic protease inhibitor design approach. Examples of these are shown in **Figure 3.1.**

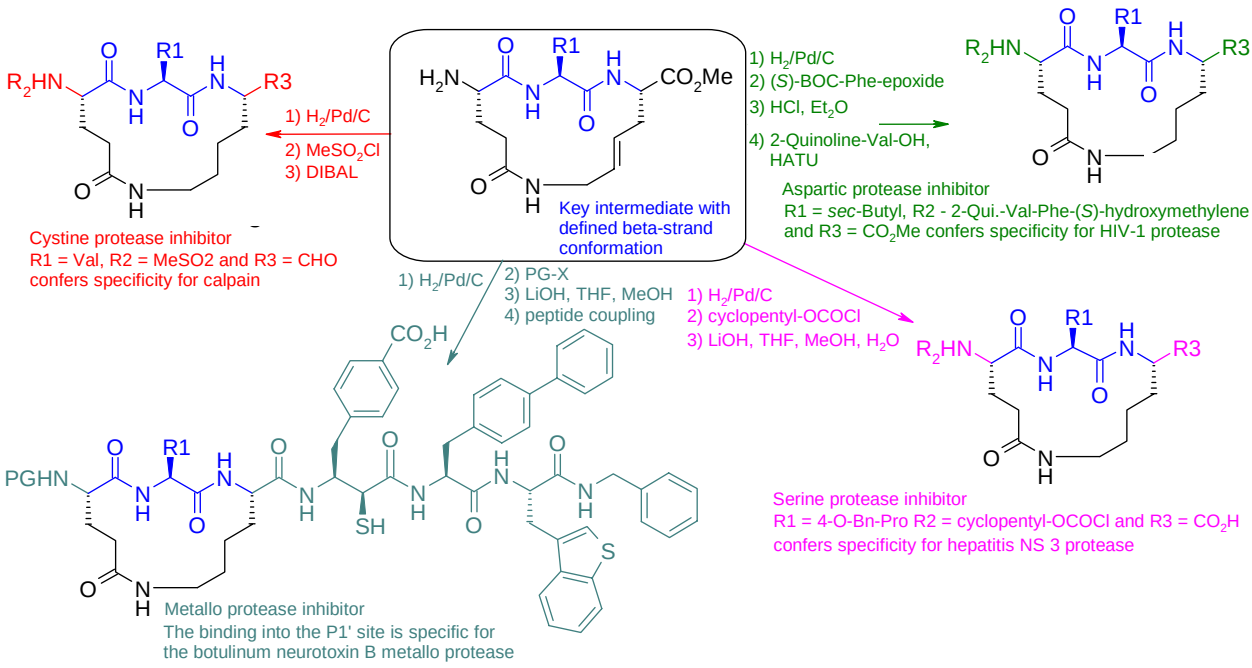


Figure 3.1: Generic protease design strategy using β-strand templates

3.2: History of RCM

The very foundation of organic synthesis consists of reactions that can reliably and efficiently form carbon-carbon bonds. Olefin metathesis is a carbon-carbon bond forming reaction which is now widely utilised in modern day organic synthesis. To many organic and polymer chemists olefin metathesis is a "new" reaction as the vast majority of research into its synthetic utility only commenced in the 1990s. Olefin metathesis was, however, first reported in the mid-1950s and in common with many catalytic reactions was discovered by serendipity while researchers were trying to expand the scope of the Ziegler and related polymerisation reactions.[4] It was Calderon and co-workers who first coined the name 'olefin metathesis' when they recognised in 1967[5] that both ring-opening polymerisation and the disproportionation of acyclic olefins were the same reaction. Today we understand this to mean the metal-catalysed redistribution of carbon-carbon double bonds. As shown in **Figure 3.2** olefin metathesis has a variety of applications. The illustrated examples include cross

metathesis (CM), ring-closing metathesis (RCM), ring-opening metathesis polymerization (ROMP) and acyclic diene metathesis polymerization (ADMET).

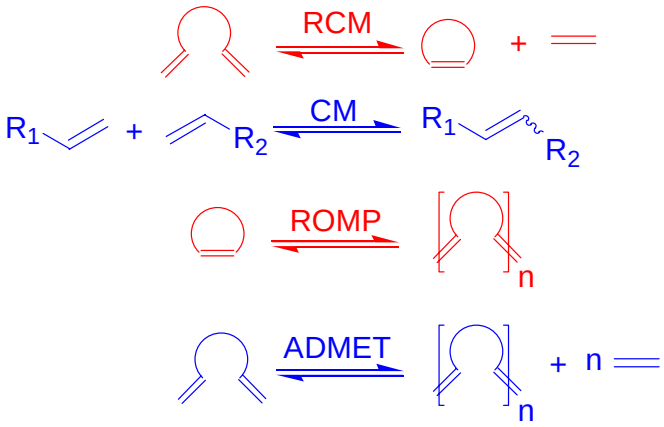


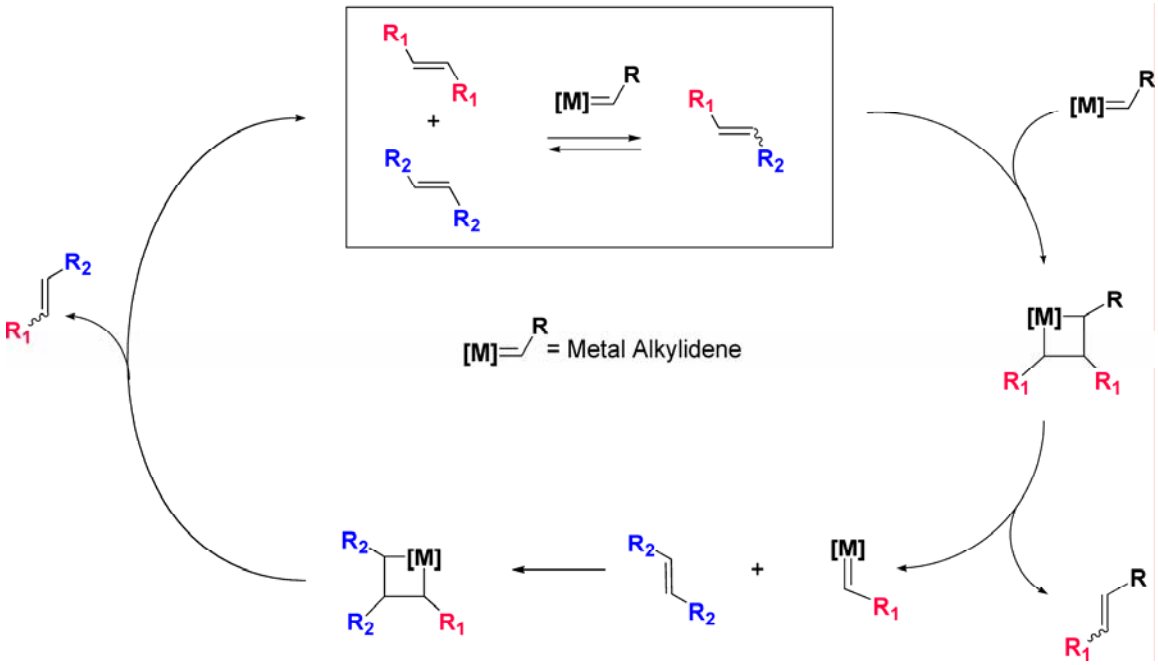
Figure 3.2: Types of olefin metathesis reactions.

3.3: Mechanism elucidation and metathesis catalyst development

In common with most other catalytic reactions, progress in the development of metathesis has been directly correlated to improvements in both catalysts and mechanistic understanding. Early metathesis catalysts from the mid-1950s to the early 1980s were poorly defined, multi-component homogeneous and heterogeneous systems consisting of transition metal salts combined with main group alkylating agents. Some of the classic combinations included WCl_6/Bu_4Sn, $WOCl_4/EtAlCl_2$, MoO_3/SiO_2, and Re_2O_7/Al_2O_3 among many others. Although these catalyst systems were able to metathesise olefins their synthetic utility was limited by the necessary harsh reaction conditions and their strongly Lewis-acidic and alkylating character. These properties rendered them incompatible with most functional groups and thus unattractive for applications in advanced organic synthesis. Furthermore these reactions were difficult to initiate and control because very little of the active species formed in the catalyst mixtures.

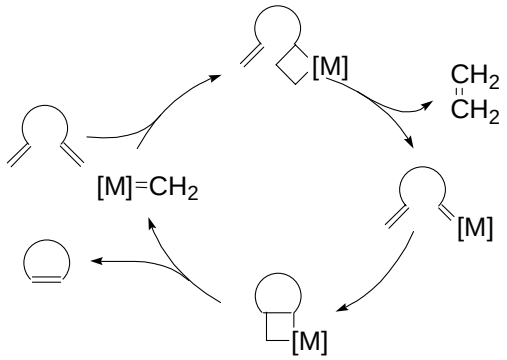
A major breakthrough in catalyst design and mechanistic understanding occurred in 1973. Researchers discovered that an unusual tantalum complex, $(Me_3CCH_2)_3Ta{=}CHCMe_3$, was the product (in quantitative yield) of the reaction between $(Me_3CCH_2)_3TaCl_2$ and two equivalents of neopentyllithium in pentane. The metal in this species appeared to be in its highest possible oxidation state, with the carbene behaving as a dianion. Around this time researchers were beginning to ask questions concerning the mechanism of the olefin metathesis reaction and the nature of the active species in classical olefin metathesis systems. The lack of definitive answers to these questions motivated extensive work to better understand olefin metathesis, including detailed mechanistic studies.

Many mechanistic schemes were proposed over the years, but ultimately, the scheme developed by Chauvin was found to be the most consistent with the experimental evidence, and it remains the generally accepted mechanism today.[6-13] As shown in **Scheme 3.1**, Chauvin proposed that olefin metathesis consists of a sequence of formal [2+2] cycloadditions/cycloreversions involving alkenes, metal carbenes, and metallacyclobutane intermediates. It is important to note that this process is reversible and therefore an equilibrium mixture of olefins is obtained. As such it is necessary to shift this equilibrium in one direction in order to make metathesis productive in preparative terms.



Scheme 3.1: Mechanism of olefin metathesis.

In the case of RCM the forward reaction is usually entropically driven. RCM cuts one substrate molecule into two products thus if one of them is volatile (e.g. ethene, propene, etc.) the desired cycloalkene accumulates in the reaction mixture (**Scheme 3.2**). Furthermore another important factor for productive RCM is the sensitivity of most metathesis catalysts to the substitution pattern of the olefin as this constitutes a kinetic obstacle for the retro-reaction.



Scheme 3.2: Mechanism of ring closing metathesis.

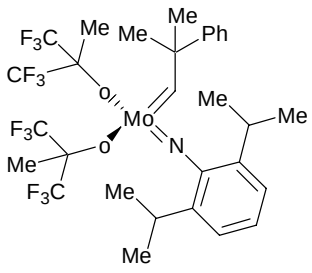
3.4: The advent of well defined single-component catalysts

The Chauvin mechanism was not only a breakthrough in terms of the mechanistic understanding of metathesis reactions but perhaps even more importantly it provided both a design rationale and a way to begin to understand catalyst activity. Subsequent efforts to

synthesise alkylidene and metallacyclobutane complexes led to the discovery of the first of many single-component homogeneous catalysts for olefin metathesis during the late 1970s and early 1980s. By 1980 investigations of tantalum alkylidene chemistry revealed that the presence of alkoxide ligands allowed tantalum species to function as metathesis catalysts for internal olefins.[14] At about the same time, oxo-alkylidene complexes of tungsten were discovered,[15] followed two years later by what turned out to be oxo-free cationic tungsten alkylidene complexes,[16] both of which would metathesise olefins. These new catalysts included $(CO)_5W{=}CPh_2$,[17, 18] bis(cyclopentadienyl)titanocyclobutanes,[19] tris(aryloxide)tantala-cyclo-butanes,[20] and various dihalo-alkoxide-alkylidene complexes of tungsten.[21-23] As well defined complexes, these catalysts exhibited better initiation and higher activity under milder conditions than ever before, thus enabling researchers to study structure-activity relationships and mechanism in detail.

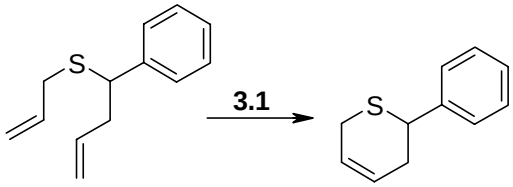
The further development of well-defined olefin metathesis catalysts was considerably aided when synthetic routes were perfected that produced electron-deficient imidoalkylidene complexes of the type $(NAr)(OR')_2M{=}CHR$ (M=Mo or W). These fully functional olefin metathesis (pre)catalysts were the first catalysts to become widely used. The ability to vary the (always sterically demanding) imido and alkoxide groups allowed a wide range of catalysts to be synthesised.[24-26] The strongly electron-withdrawing hexafluoro-tert-butoxide ligand was found to result in the most rapid turnover. The commercial availability of the molybdenum hexafluoro-tert-butoxide complex, which is now commonly recognised as Schrocks catalyst, **3.1**, as well as its speed and selectivity resulted in it being explored for organic transformations.

For a considerable period of time **3.1** was the only metathesis catalyst that allowed the formation of tri- and even tetrasubstituted double bonds by RCM. Only recently was a ruthenium-based system developed which also allowed access to these products.[27]



3.1

An additional advantage of the molybdenum complex **3.1** resides in its tolerance towards certain functional groups which inhibit ruthenium-based metathesis catalysts. Typically sulfur and phosphine containing compounds can be problematic. A mismatch of the hard Mo^{VI} centre with soft sulfur or phosphine functionalities can explain why substrates such as **3.2**, shown in **Scheme 3.3**, cyclise smoothly in the presence of **3.1** but usually fail to react in the presence of ruthenium catalysts.[28-32]



3.2

Scheme 3.3: Functional group tolerance of **3.1**

Furthermore, catalyst **3.1** is little affected by the electronic properties of the olefinic substrates and reacts with both electron-rich olefins (for example, enol ethers) and electron-poor olefins (such as acrylates and acrylonitrile).[33-38] Until very recently the ruthenium catalysts were mostly unreactive towards these substrates,[39] but newly developed heteroleptic ruthenium species have largely overcome this problem.

The major shortcoming of the molybdenum catalysts, and others based on the early transition metals, is the high oxophilicity of the metal centre which renders them extremely sensitive to oxygen and moisture. As an example, the synthesis and handling of **3.1** requires an inert atmosphere and rigorously purified, dried, and degassed solvents/reagents. In addition early metal catalysts are more fundamentally limited by moderate to poor functional group

tolerance. Complex **3.1**, for instance, is incompatible with aldehydes and alcohols.[30] This problem of functional group tolerance may be overcome with protecting group strategies, but these are often tedious, time consuming, and synthetically inefficient. The development of single-component catalysts, and in particular the Schrock type of catalysts, was a major advance but significant room for improvement remained. Continuing research was motivated by the prospect of solving the problems related to oxophilicity and functional group tolerance.

First generation ruthenium catalysts

The key to improved functional group tolerance in olefin metathesis was the development of a catalyst that reacted preferentially with olefins in the presence of heteroatomic functionalities. A very important conclusion derived from the early work on catalyst design was that the catalysts were observed to react more selectively with olefins as the metal centres were varied from left to right and bottom to top in the periodic table.[40] This trend is illustrated for titanium, tungsten, molybdenum, and ruthenium in **Figure 3.3.**

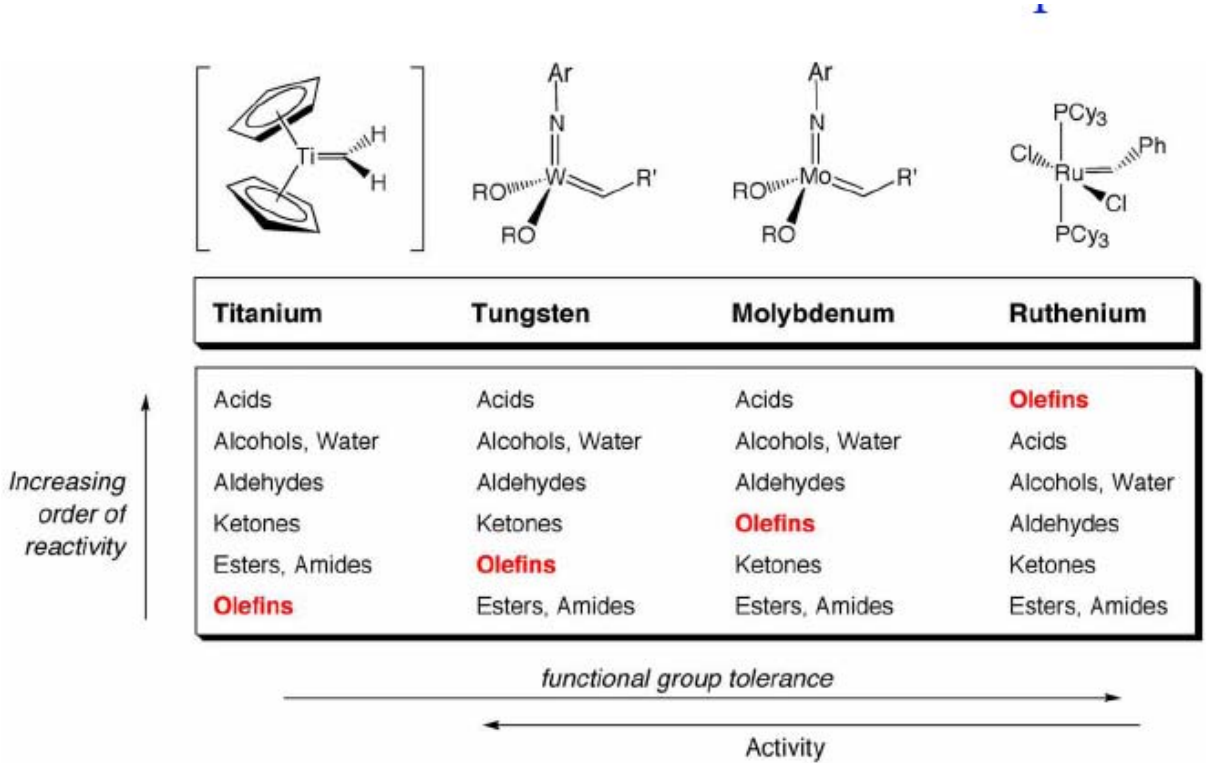
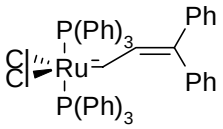


Figure 3.3: Functional group tolerance of early and late transition metal catalysts.

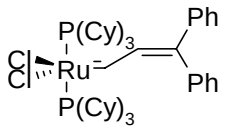
In retrospect, ruthenium based catalysts were excellent candidates as metathesis catalysts but these were not seriously considered for more than two decades. This was the result of discouragingly low metathesis activity of ruthenium salts, as well as a limited understanding of how to achieve functional group tolerance. Several reports from the 1960s had, in fact, described the ROMP of norbornene derivatives with $RuCl_3$(hydrate) in refluxing ethanol under aqueous emulsion conditions.[41, 42] The polymer yields were small, but the observation that ROMP could take place in these protic solvents at all was an important precedent.

A breakthrough occurred when **3.3** (as a mixture of *cis*- and *trans*-bis(phosphine) isomers) was successfully synthesised. This was the first well-defined, metathesis-active ruthenium alkylidene complex.[43]



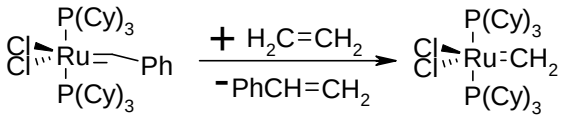
3.3

Although the initiation behaviour and functional group tolerance of **3.3** was encouraging, its activity was limited to the ROMP of highly strained monomers. To extend this activity to the metathesis of acyclic olefins, the influence of the ligands on the catalytic activity of 5-coordinate, 16-electron, ruthenium complexes were systematically studied. From these studies it was concluded that, with regard to the anionic substituent, chloride seemed to be optimal. The effect of this rather electron-withdrawing group, however, must be counterbalanced by electron-donating phosphines. Based on the trend followed by early transition metal catalysts, for which metathesis activity increases with more electron-withdrawing ligands,[44, 45] a variety of cationic complexes and derivatives containing less basic phosphines were prepared and tested. This culminated in the discovery that the larger and more basic the phosphine, the higher the metathesis activity. The tricyclohexylphosphine derivative (**3.4**) was the first ruthenium alkylidene complex active toward acyclic olefins.



3.4

While this catalyst is not as active as the molybdenum complex **3.1** it is more versatile due to its improved functional group tolerance. For example, it was shown to cyclise dienes to five-, six-, and seven-membered carbocycles and heterocycles in good yields, even in the presence of trifluoroacetyl and *tert*-butoxycarbonyl, protecting groups.[46] In addition, it is air-stable as a solid and it retains its activity even when exposed to water, alcohols, or acids. These characteristics made catalyst **3.4** an ideal starting point for further catalyst optimisation. With a variety of complexes prepared, the fundamental reactions of ruthenium alkylidenes were examined. The benzylidene catalyst **3.5**, was found to undergo metathesis with ethylene within minutes at room temperature to quantitatively form the methylidene derivative as shown in **Scheme 3.4**. This complex was the first metathesis-active methylidene species ever isolated.



3.5

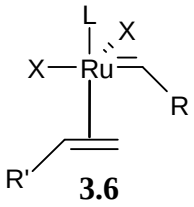
Scheme 3.4: Metathesis of ethylene with catalyst **3.5**

Catalyst **3.5** combined the bulky and strongly electron-donating $P(Cy)_3$ ligands with the readily initiated benzylidene moiety and this complex is now commonly referred to as Grubbs first generation catalyst.

Second generation ruthenium catalysts

Many mechanistic studies were performed using the first generation ruthenium catalysts. The key insight was that **3.5** formed a highly active mono-phosphine intermediate during the

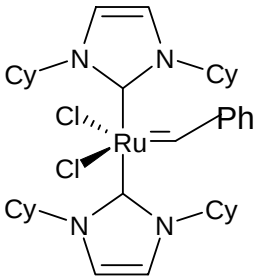
catalytic cycle. As such this intermediate became a design motif (**3.6)** which was used as a starting point for the development of improved catalysts.



3.6

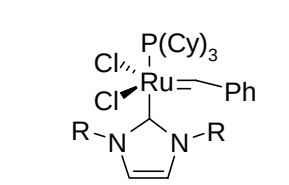
Furthermore, as the decomposition of **3.5** was known to be second order and inversely proportional to phosphine concentration, it was important to maintain a low concentration of the mono-phosphine species. As such it is important that the ancillary ligands are not too labile and that they sufficiently stabilised the reaction intermediates. Any approach to increase the concentration of the mono-phosphine intermediate would be futile if it also accelerated catalyst decomposition. It was therefore concluded that the steric and electronic properties of the residual neutral ligands were decisive for the performance of the catalyst. It was postulated improved catalytic activity would be achieved if these ligands were even more basic and sterically demanding than $P(Cy)_3$.

At the time when this work was being undertaken it was already known that, compared to phosphines, *N*-heterocyclic carbene ligands were both stronger donors and much less labile.[47] The first report on their use in the context of metathesis, [48] in which both $P(Cy)_3$ ligands of **3.5** were replaced by *N*,*N*-disubstituted 2,3-dihydro-1*H*-imidazol-2-ylidene moieties **3.7**, proved to be a rather stable complex and hence it did not exhibit an improved activity profile over **3.5**. This result was a mechanistically reassuring discovery since the "sticky" NHC ligands rendered the dissociation of the ligand less likely and thus resulted in a low concentration of the catalytically active ruthenium template in solution and hence poorer catalytic activity.



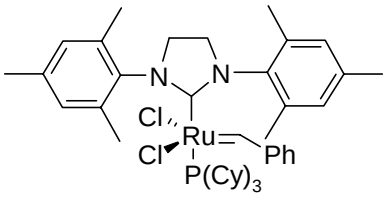
3.7

The use of a mixed-ligand complex overcame this problem. Incorporation of one kinetically inert, electron-donating NHC ligand in combination with a coordinatively labile ligand resulted in the desired synergetic effect. This hypothesis was independently pursued by three different research groups, each of which reported[49-57] almost simultaneously on the preparation and the catalytic properties of the heteroleptic complexes shown in **Figure 3.4**.

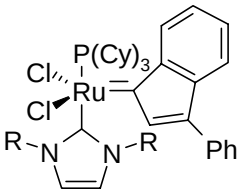


R = Mesityl or CHMePh

3.8

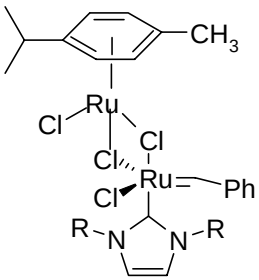


3.9



R = Mesityl

3.10



R = C_6H_{11}

3.11

Figure 3.4: Ruthenium heteroleptic complexes.

These second-generation ruthenium carbene catalysts displayed performance that was previously only possible with the most active early metal systems. These also possessed the thermal stability and the resistance toward oxygen and moisture, as well as the compatibility with many functional groups previously observed with the first generation of ruthenium catalysts. As such these second generation catalysts were most significant because they

combined the best characteristics of early and late metal centres into a single species. Until then it appeared that functional group tolerance was only gained at the expense of activity. An example of this "leap forward" in catalytic activity is evidenced with the ease with which the RCM of sterically demanding dienes of tri- and tetrasubstituted olefins was performed.[49-55] Similarly, electron deficient olefins which constituted problematic substrates for the first generation ruthenium catalysts can be ring closed using the second generation ruthenium catalysts.[54, 58]

The major milestones achieved during the development of RCM methodology are illustrated in **Figure 3.5**. The work contained in this thesis is focussed upon attempting to incorporate and extend RCM methodology into the synthesis of conformationally constrained β-strand mimics.

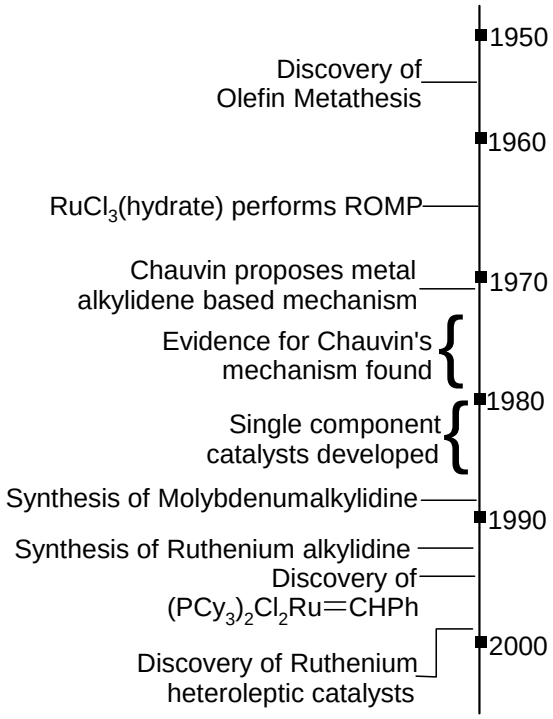
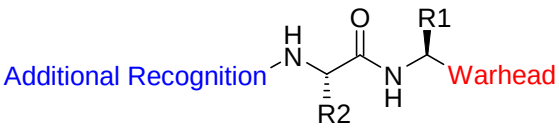


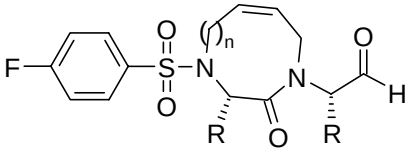
Figure 3.5: Major milestones achieved during the development of RCM methodology.

3.5: Design and synthesis of N-N conformationally constrained SJA analogues

The first strategy envisaged to conformationally constrain compounds of type **2.7**, into a β-strand, using RCM was to introduce a conformational constraint between two adjacent amino acids to form a *N-N* cyclised ring of variable size (**Figure 3.6**).



2.7



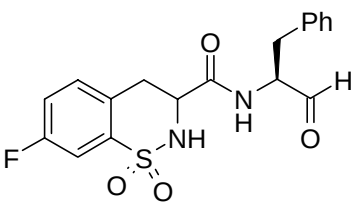
3.12

Figure 3.6: *N-N* cyclised analogue of **2.7**

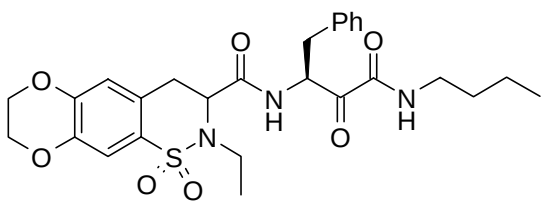
The decision to design and synthesise the *N-N* cyclised analogues of the general structure **3.12** was motivated by two recent publications.

- Carbocycles of type **3.12** were shown to have a reduced conformational space that results in an increased affinity for biological receptors. In addition this cyclisation confers dramatically improved redox stability.[59-62]

- In 2004 researchers at Cephalon published a paper[63] containing interesting SAR around related types of compounds as calpain I inhibitors. Structure **3.13,** with an IC_{50} of 7 nM, indicated that a conformationally constrained sulfonamide could be used to obtain potent compounds. Molecular modeling in our group, by Blair Stuart, indicated that compounds of this type bound to calpain in β-strand conformation (**Figure 3.7**). In addition, structure **3.14** with an IC_{50} of 50 nM indicated that if the correct conformational constraint was chosen the NH of the sulfonamide could be alkylated without significant loss of protease inhibition.



3.13



3.14

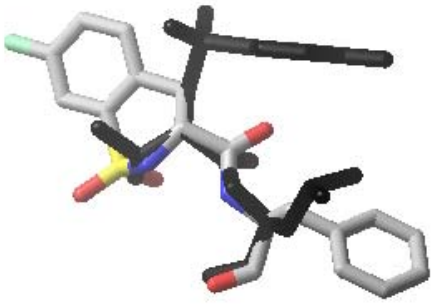
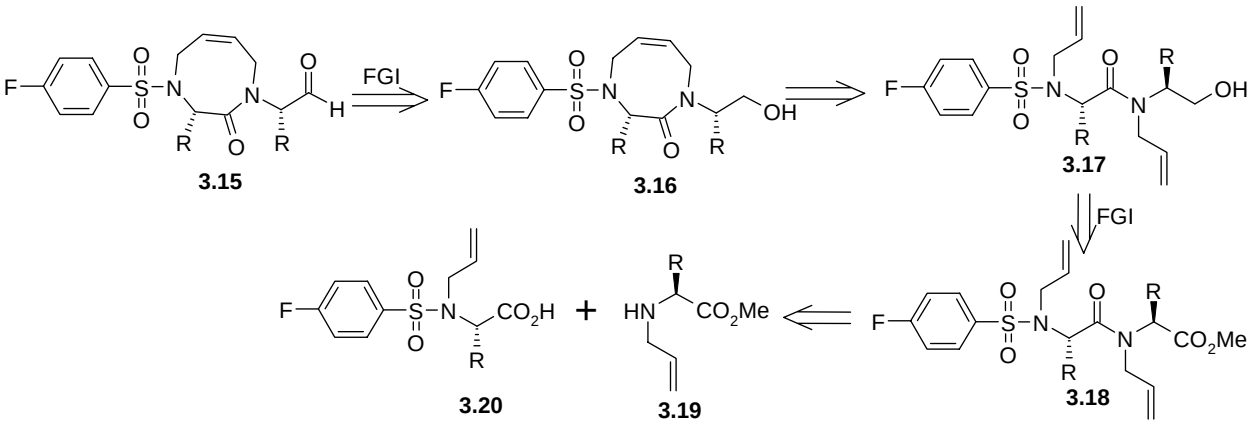


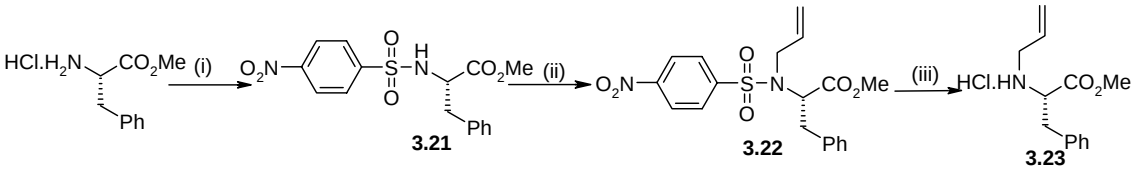
Figure 3.7: Superimposition of **2.12** (black) and **3.13** (CPK colours)

Retrosynthetic analysis (**Scheme 3.5**) was used to decide on the synthetic route to compounds of type **3.12**. The key disconnections were that of cyclised alcohol **3.16** to diene **3.17** and the disconnection of diene **3.18** to its constituent carboxylic acid and amine (**3.19** and **3.20**).



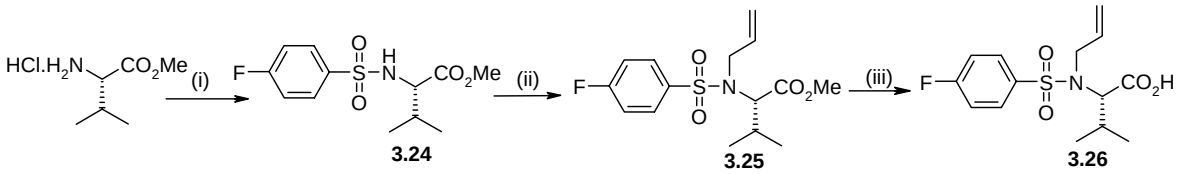
Scheme 3.5: Retrosynthetic analysis of **3.15**

Phenylalanine was chosen as the P1 substituent and valine as P2. The synthesis of required amine **3.19** is shown in **Scheme 3.6**. Phenylalanine methyl ester was activated towards *N*-allylation using a procedure established by Reichwein and Liskamp[60]. This was achieved by formation of 4-nitrobenzene sulfonamide **3.21**. This was allylated using mildly basic conditions to yield *N*-allylated sulfonamide **3.22**. The *N*-activating sulfonamide of **3.22** was cleaved using thiophenol to afford required *N*-allyl amine **3.23**.



Scheme 3.6. *Reagents and Conditions:* **(i)** 4-Nitrobenzene sulfonyl chloride, Et_3N, DCM, (81%); **(ii)** K_2CO_3, allyl bromide, DMF, (93%); **(iii)** K_2CO_3, thiophenol, DMF, (65%)

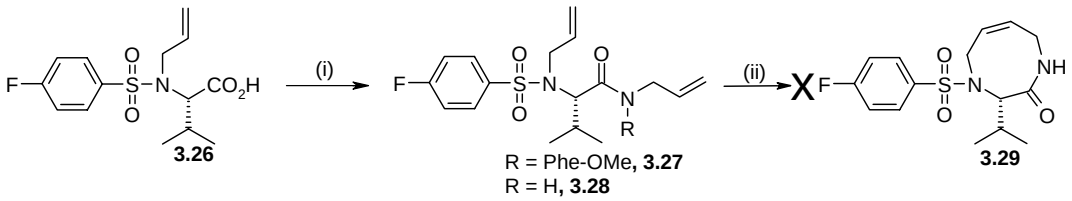
The preparation of required carboxylic acid **3.26** is shown in **Scheme 3.7**. Valine methyl ester was coupled with 4-fluorobenzene sulfonyl chloride to yield sulfonamide **3.24**. This was allylated using potassium carbonate as the base and the ester hydrolysed to form desired *N*-allyl carboxylic acid **3.26**.



Scheme 3.7. *Reagents and Conditions:* **(i)** 4-Fluorobenzene sulfonyl chloride, DIPEA, DCM (92%); **(ii)** K_2CO_3, allyl bromide, DMF, (89%); **(iii)** NaOH, THF, H_2O, MeOH, (95%)

As shown in **Scheme 3.8** coupling of *N*-allyl amine **3.23** and *N*-allyl carboxylic acid **3.26** was attempted using a wide variety of peptide coupling reagents. All these failed. In addition the acid chloride and acid fluoride of **3.26** were prepared, but these both failed to form desired di-peptide **3.27** on reaction with amine **3.23**. As such this synthetic route was abandoned.

An alternative route to the target compound was devised when previously prepared *N*-allyl carboxylic acid **3.26** was coupled with allyl amine to yield diene **3.28** (**Scheme 3.8**). This was subjected to Grubbs second generation catalyst under a variety of reaction conditions but no ring closed product was obtained. Only starting diene **3.28** was recovered in quantitative yield.. As such this synthesis was also not pursued further.



Scheme 3.8. *Reagents and Conditions:* ***For R = Phe-OMe***: (i) HATU, DIPEA, **3.23**, DMF; or HATU, DIPEA, **3.23**, THF; or HATU, DIPEA, **3.23**, (1/1) DMF/DCM; or EDC, HOAT, DIPEA, **3.23**, DMF; or PyAOP, DIPEA, **3.23**, DMF; or PyBroP, DIPEA, **3.23**, DMF; or a) Cyanuric fluoride, Pyr, DCM; b) **3.23**, DCM; or a) $(COCl)_2$, DMF, DCM b) **3.23**, DCM (all 0%). **For R = H:** (i) HATU, HOAt, DIPEA, allyl amine, DMF, (73%); b) 10 mol% **3.9,** DCM, rt; or 10 mol% **3.9;** benzene, reflux; or 10 mol% **3.9;** 1,1,2-TCE, reflux; or 3x 10 mol% **3.9;** 1,1,2-TCE, microwave (all 0%)

The reason for the failure of diene **3.28** to undergo RCM is postulated to be due to the diene forming a stable six membered catalyst deactivating chelate with Grubbs second generation catalyst (**Figure 3.8**) (discussed in further detail in **Section 4.16**).

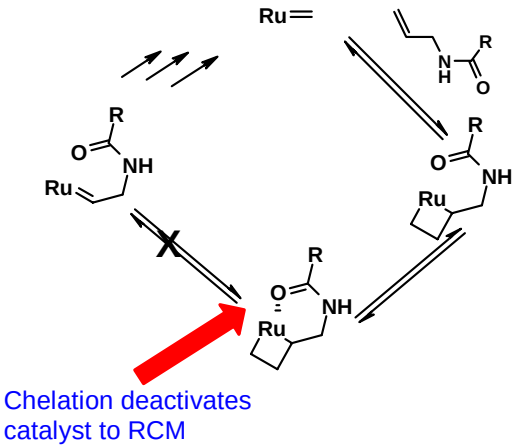
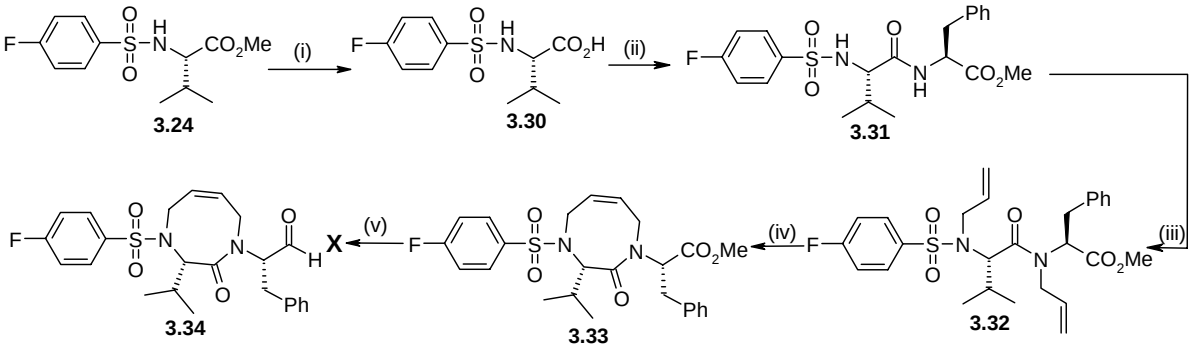


Figure 3.8: Chelate formation from **3.28** and Grubbs second generation catalyst

The synthetic route, therefore, had to be modified to negate RCM failure due to chelation and an inability to couple *N*-allyl amine **3.23** with *N*-allyl carboxylic acid **3.26**. As discussed further in **Section 4.16** the chelate is particularly strongly bound when a proton is located on the amide. However, as it was not possible to couple an *N*-allyl amine the allyl group had to be introduced after peptide coupling. As shown in **Scheme 3.9** this was achieved using P_4-

phosphazene. Methyl ester **3.24** was hydrolysed with sodium hydroxide and resultant carboxylic acid **3.30** was coupled with phenylalanine methyl ester to afford di-peptide **3.31**. A double allylation was achieved using allyl bromide and P_4-phosphazene to yield diene **3.32**. RCM was accomplished using Grubbs second generation catalyst in refluxing 1,1,2-TCE in 60% isolated yield. Reduction of tetrahydro-diazocinone methyl ester **3.33** with DIBAL-H resulted in a complex mixture of inseparable products**.** As such this synthetic sequence was abandoned.

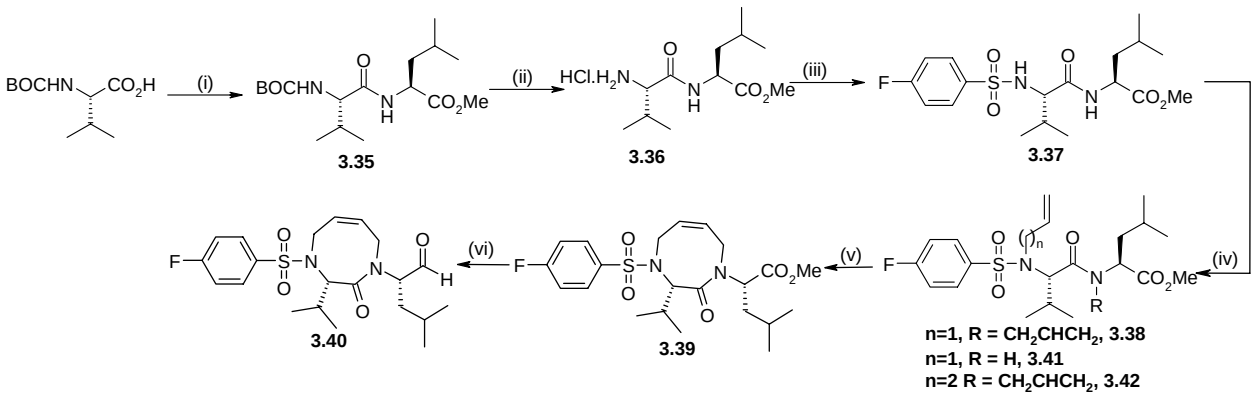


Scheme 3.9. *Reagents and Conditions:* **(i)** LiOH.H_2O, MeOH, H_2O, (100%); **(ii)** HATU, HOAt, DIPEA, Phe-OMe, DMF, (86%); **(iii)** P_4-Phosphazene, allyl bromide, THF, (51%); **(iv)** 3x 10 mol%, 1,1,2-TCE, microwave, (60%) **(v)** DIBAL-H, DCM (0%)

As shown in **Scheme 3.10** tetrahydro-diazocinone methyl ester **3.39** was prepared using the same synthetic methodology as that used in **Scheme 3.9.** The only difference was the incorporation of valine as the P2 residue. This was introduced in order to obtain directly comparable SAR with acyclic analogue **2.12**. However, reduction of methyl ester **3.39** with lithium aluminium hydride also resulted in complete decomposition. Moreover around the same period of time advances in molecular modelling suggested that this class of potential inhibitors would provide poor β-strand mimics and as such the synthesis of these types of compounds was terminated.

Despite this setback the synthetic methodology devised in **Schemes 3.9** and **3.10** presented an opportunity to develop methodology for the facile variation of ring size. The important

observation was that the proton of the sulfonamide was more acidic than that of the amide. It was envisaged that a selective allylation could be achieved using a mild base. As shown in **Scheme 3.10** dipeptide **3.37** was allylated with both allyl bromide and 4-bromo-but-1-ene, using potassium carbonate as base, to yield mono-allylated dipeptides **3.41** and **3.42**. No further work was performed on these types of compounds as they were rated as low priority after the successful synthesis of the β-strand macrocycles (see **Chapters 4 and 5**). However allylation of the amide proton of **3.41** and **3.42** using P_4-phosphazene base and either allyl bromide or 4-bromo-but-1-ene respectively would yield nine and ten membered rings after RCM.

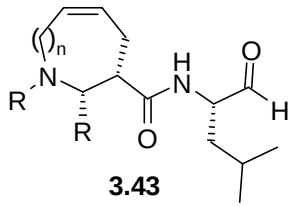


Scheme 3.10. *Reagents and Conditions*: **(i)** HATU, DIPEA, Leu-OMe, DMF, (93%); **(ii)** 2M HCl, Et_2O, (100%); **(iii)** 4-Fluorobenzene sulfonyl chloride, DIPEA, DCM (24%);
For n = 1, R = CH_2CHCH_2: (iv) P4-phosphazene, allyl bromide, THF (37%); **(v)** 3x 10 mol%, **3.9**, 10 mol% $(Cy)_2BCl$, 1,1,2-TCE, microwave, (60%); (vi) $LiAlH_4$, THF, (0%)
For n = 1, R = H: (iv) K_2CO_3, allyl bromide, DMF, (78%); **For n = 2, R = H: (iv)** K_2CO_3, 4-bromo-1-butene, DMF, (74%)

3.6: Design and synthesis of β-amino acid C-N carbocycles

Previous work in our laboratory systematically replaced the leucine and valine α-amino acids in **2.12** with β-amino acids.[64] However, all the compounds prepared in this series proved to have low activity. From this work it was concluded that incorporation of β-amino acids into a calpain inhibitor was detrimental to inhibitory activity.

However, as β-amino acids are known to increase the biostability of peptide-based drugs[65] it was postulated that, if it was possible to conformationally constrain β-amino acid compounds into a β-strand then this might provide an approach for the design and synthesis of metabolically stable β-strand mimics. As such general structure **3.43** was selected as a synthetic target.



3.43

Molecular modelling (**Figure 3.9**) of **3.43** where n is 1 and the R substituent is a 4-fluorobenzene sulfonamide indicated a β-strand conformation may be obtained. It was not a perfect β-strand mimic but it was a good starting point for RCM studies in these types of compounds. Furthermore molecular modelling indicated that the aldehyde was located in close proximity to the catalytic cysteine residue.

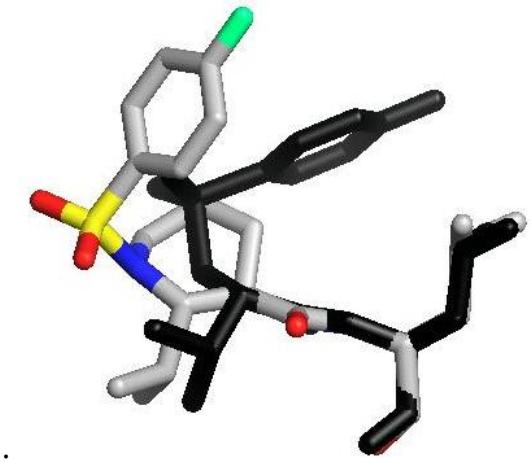
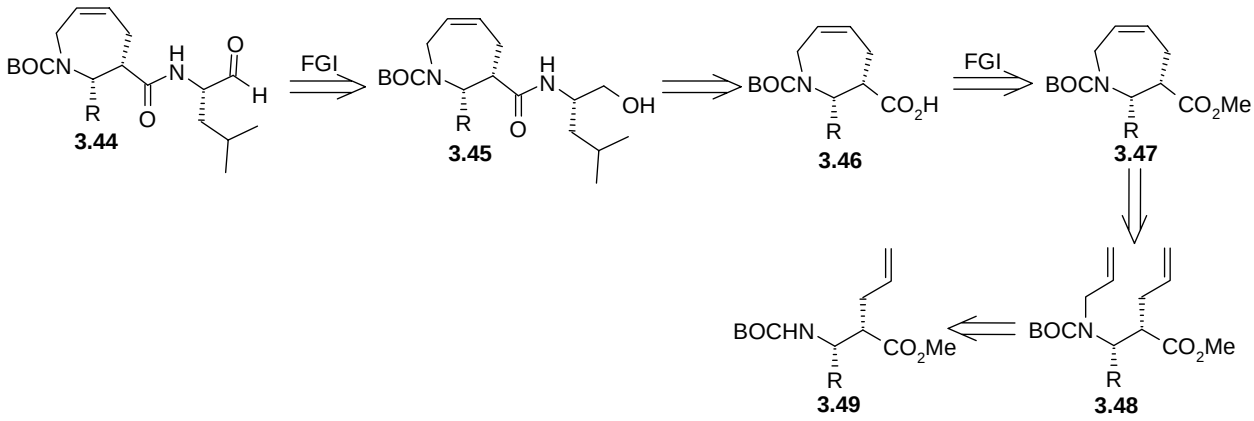


Figure 3.9: Superimposition of **2.12** (Black) and **3.43** R = 4-F-phenyl sulfonamide and n = 1 (CPK colours)

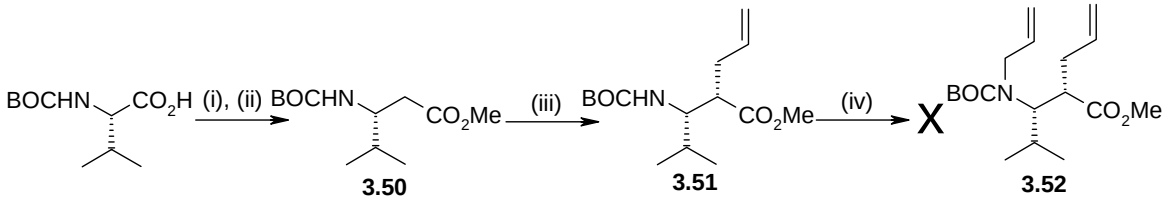
One of the objectives of the synthesis was to develop versatile synthetic methodology that would allow a variety *N*-substituted carbocycles to be prepared. Retrosynthetic analysis (**Scheme 3.11**) of **3.43** incorporating the easily cleaved *N*-BOC protecting group was performed. A further consideration factored into the retrosynthesis was that it was considered

prudent to synthesise the conformational constraint first in order to establish that ring closure using RCM could be achieved.



Scheme 3.11: Retrosynthetic analysis of **3.44**

As shown in **Scheme 3.12** valine was chosen as the P2 residue as this generally results in the most potent calpain inhibition.[66] As per the retrosynthetic analysis (**Scheme 3.11**), *N*-BOC-Val-H was converted to *N*-BOC-β-Val-OMe (**3.50**) using Arndt-Eistert homologation conditions.[67] Allylation was achieved, albeit it in low yield, to afford **3.51**. However, *N*-allylation of this was not possible using allyl bromide and sodium hydride as the base. It was decided that this failure, combined with the low yield and problematic purification of **3.51,** required the design of an improved synthetic route.

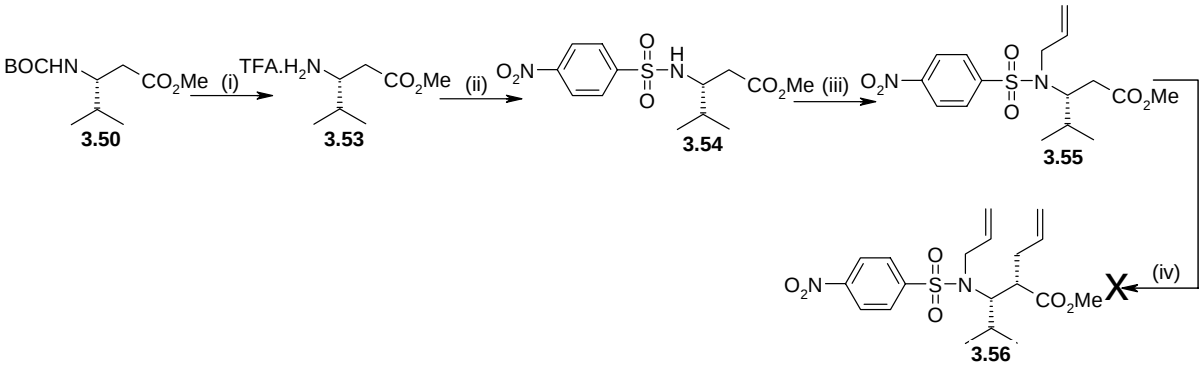


Scheme 3.12. *Reagents and Conditions*: **(i)** $ClCO_2Et$, Et_3N, THF; **(ii)** CH_2N_2, AgOBn, Et_3N, MeOH, (65% over 2 steps); **(iii)** LDA, LiCl, allyl bromide, THF -78^oC, (16%) **(iv)** NaH, allyl bromide, THF, (0%)

The new methodology had to provide both ease of *N*-allylation and C-allylation. It was postulated that the NH proton of **3.50** may have contributed to the side products obtained when this was C-allylated using LDA and allyl bromide. As such it was hypothesised the use of a 4-nitrobenzene sulfonamide would not only activate the nitrogen towards allylation but *N*-allylation would also remove the proton from the sulfonamide. As such it was envisaged

that using this strategy, cleaner higher yielding C-allylation would be achieved. This methodology was therefore incorporated into a revised synthetic strategy.

As shown in **Scheme 3.13** the BOC protecting group of *N*-BOC-β-valine (**3.50),** prepared earlier (see **Scheme 3.12**), was cleaved using a 10% solution of TFA in DCM to afford amine **3.53**. Sulfonamide **3.54** was prepared using standard sulfonyl chloride coupling conditions. This was allylated with allyl bromide using potassium carbonate as the base to give *N*-allyl-β-amino acid **3.55**. However, C-allylation using either LDA or LiHMDS as base resulted in complete decomposition. This synthetic route was therefore abandoned.

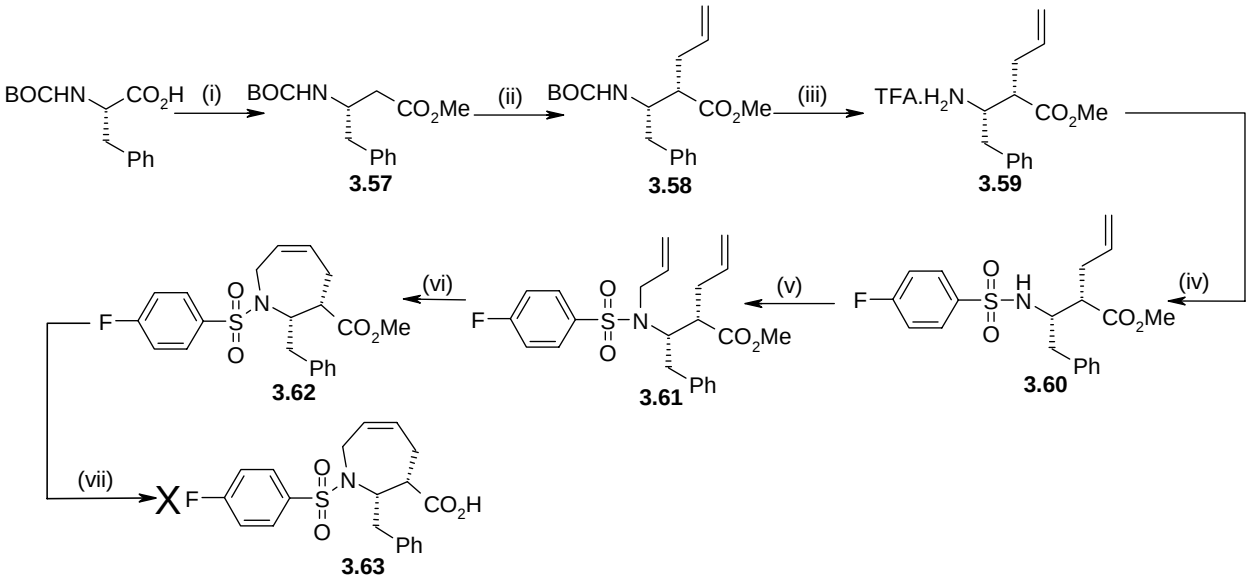


Scheme 3.13. *Reagents and Conditions*: **(i)** 20% TFA, DCM, (100%); **(ii)** 4-Nitrobenzene sulfonyl chloride, Et_3N, DCM, (30%); **(iii)** K_2CO_3, allyl bromide, DMF, (100%); **(iv)** LDA, LiCl, allyl bromide, THF; or LiHMDS, allyl bromide, THF, (both 0%)

As valine was proving difficult to work with due to problems with chromatographic purification, particularly given the complex mixture of products obtained, the amino acid was changed to phenylalanine.

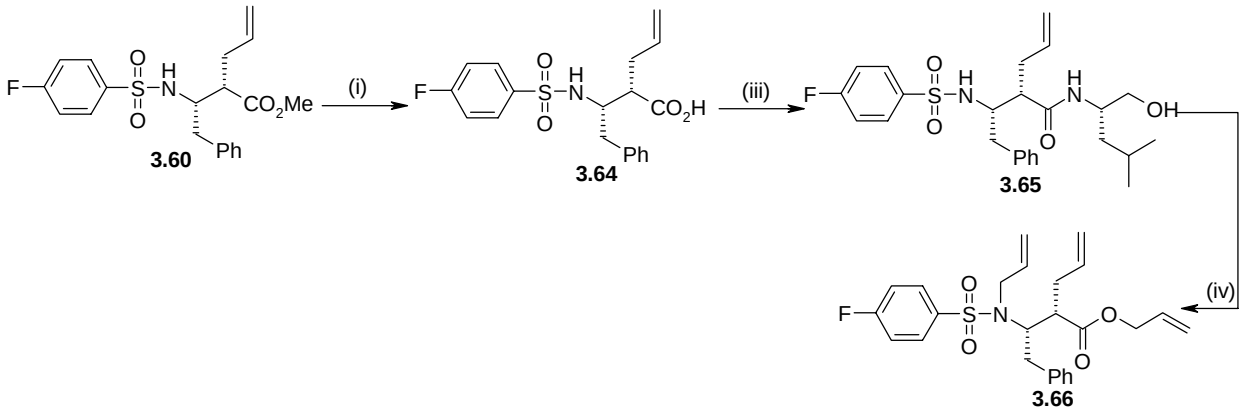
As shown in **Scheme 3.14** synthesis of carbocycle **3.62** was successfully achieved using phenylalanine as the amino acid and 4-fluorobenzene sulfonyl chloride as an *N*-activating group. BOC protected phenylalanine was converted to β-amino acid methyl ester **3.57** using Arndt-Eistert homologation conditions.[67] Stereoselective allylation was achieved, albeit in low yield to afford **3.58**. This reaction was performed on a ten gram scale to ensure that sufficient material was obtained to continue the synthesis. The BOC protecting group was cleaved using TFA in DCM to give the TFA salt of amine **3.59**. Treatment of this under

standard sulfonyl chloride coupling conditions with 4-flourobenzene sulfonyl chloride afforded sulfonamide **3.60**. This was allylated using mildly basic conditions to afford diene **3.61**. This was ring closed using Grubbs second generation catalyst in DCM at room temperature to give cyclised product **3.62** in 81% isolated yield. However, under basic hydrolysis conditions **3.62** decomposed.



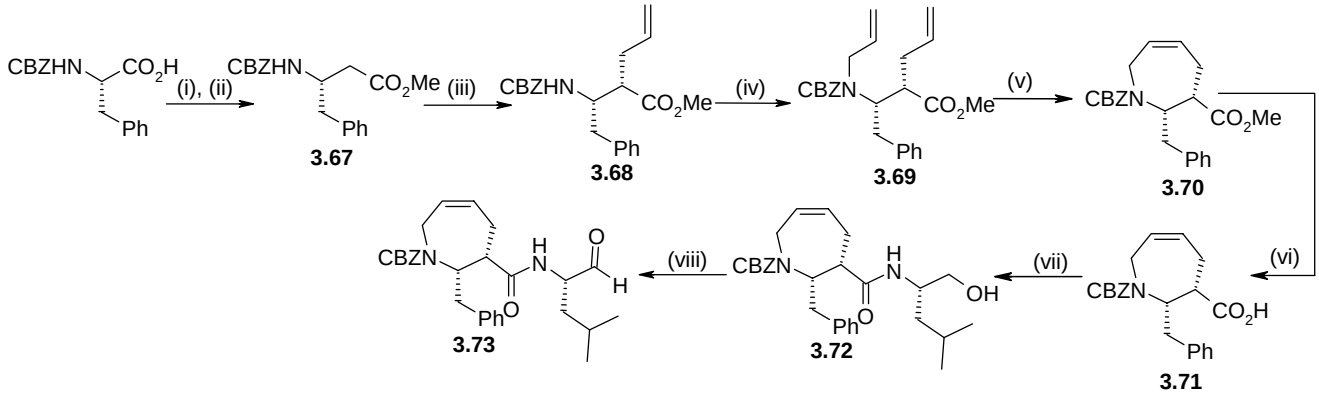
Scheme 3.14. *Reagents and Conditions*: **(i) a)** $ClCO_2Et$, Et_3N, THF; **b)** CH_2N_2, AgOBn, Et_3N, MeOH, (62% over 2 steps); **(ii)** LDA, LiCl, allyl bromide, THF -78^oC, (26%) **(iii)** 10% TFA, DCM (100%); **(iv)** 4-Fluorobenzene sulfonyl chloride, Et_3N, DCM, (100%); **(v)** K_2CO_3, allyl bromide, DMF, (82%); **(vi)** 10 mol% **3.9**, DCM (81%); **(vii)** $LiOH.H_2O$, MeOH, H_2O, THF, (0%)

In order to circumnavigate the base sensitivity of cyclised ester **3.62**, an alternative order of synthetic steps was attempted in which hydrolysis of methyl ester **3.60** was performed much earlier in the synthetic sequence (**Scheme 3.15**). Resultant carboxylic acid **3.64** was coupled with (*L*)-leucinol using standard peptide coupling procedures to form dipeptide **3.65.** However, allylation under standard mild base conditions, developed earlier in this section, resulted in triolefinic product **3.66.** This was an unexpected and unexplained product, however NMR and mass spectrometry characterisation confirmed this to be the structure.



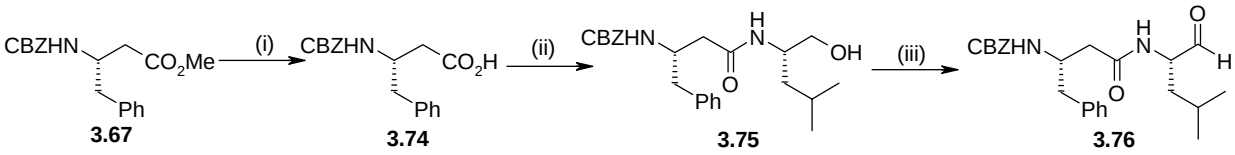
Scheme 3.15. *Reagents and Conditions*: **(i)** LiOH, MeOH, H_2O, THF, (46%); **(ii)** EDC, HOAt, DIPEA, (*L*)-leucinol, DMF, (61%); **(iii)** K_2CO_3, allyl bromide, THF, (84%), **(iv)** LDA, LiCl, allyl bromide, THF -78^oC

Required aldehyde, **3.73**, was successfully prepared using the synthetic sequence shown in **Scheme 3.16.** Arndt-Eistert homologation of *N*-CBZ-Phe-H afforded β-amino acid **3.67**. Stereoselective C-allylation using LDA as base gave olefin **3.68** which was *N*-allylated using P_4-phosphazene base to give diene **3.69**. Treatment with Grubbs second generation catalyst then gave **3.70**. Hydrolysis of the methyl ester with sodium hydroxide, followed by peptide coupling of carboxylic acid **3.71** with (*L*)-leucinol afforded dipeptide alcohol **3.72**. Oxidation with sulfur trioxide pyridine complex in DMSO/DCM produced required aldehyde **3.73**.



Scheme 3.16. *Reagents and Conditions*: **(i)** $ClCO_2Et$, Et_3N, THF; **(ii)** CH_2N_2, AgOBn, Et_3N, MeOH, (48% over 2 steps); **(iii)** LDA, LiCl, allyl bromide, THF, (65%); **(iv)** P_4-Phosphazene, allyl bromide, THF, (87%); **(v)** 10 mol% **3.9**, DCM, (82%); **(vi)** NaOH, THF, H_2O, (100%); **(vii)** EDC, HOAt, DIPEA, (*L*)-leucinol, DMF, (58%); **(viii)** SO_3.Pyr, DIPEA, DMSO, DCM, (61%)

In order to obtain meaningful SAR the direct acyclic analogue of **3.73** was synthesised. As shown in **Scheme 3.17** this was achieved using standard peptide chemistry. Methyl ester **3.67** was hydrolysed and resultant carboxylic acid **3.74** coupled with (*L*)-leucinol to give dipeptide alcohol **3.75**. Oxidisation with sulfur trioxide pyridine complex in DMSO/DCM gave aldehyde **3.76**.



Scheme 3.17. *Reagents and Conditions*: **(i)** NaOH, THF, H_2O, MeOH, (94%); **(ii)** EDC, HOAt, DIPEA, (*L*)-leucinol, DMF, (30%); **(iii)** SO_3.Pyr, DIPEA, DMSO, DCM, (52%)

Compounds **3.72**, **3.73**, **3.75** and **3.76** were then assayed against calpain II and the results are given in **Table 3.1.**

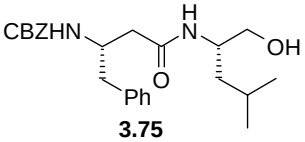
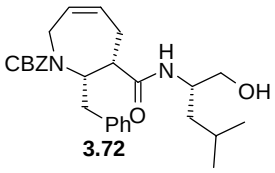
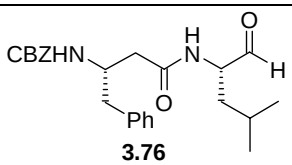
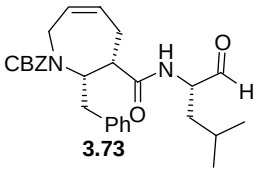
Compound	Glide Score	Number of β-strand hydrogen bonds to enzyme	Warhead distance to Cys (Å)	IC_{50} (nM)
 **3.75**	-7.3	0	3.8	228000
 **3.72**	-5.4	0	6.7	179000
 **3.76**	-6.4	0	7.2	4230
 **3.73**	-6.6	0	6.6	3150

Table 3.1: SAR of β-amino acid C-N carbocycles

These results suggest that incorporation of β-phenylalanine at P2 in the acyclic series results in only moderate calpain inhibition (see compounds **3.75** and **3.76**). This result is rationalised by molecular modelling. Inclusion of a β-amino acid into the peptide backbone results in the loss of β-strand conformation and thus the loss of β-strand hydrogen bonds in the enzyme active site.

In addition, from **Table 3.1** it can also be inferred that cyclic inhibitor **3.73** is not in the 'bio-active' conformation. This conclusion is reached from the inhibition results of **3.73** and **3.76.** The IC_{50} values for these two analogues suggest that they are equipotent. As molecular modelling clearly shows that the acyclic analogue is not able to bind in a β-strand conformation then as the cyclic analogue is equipotent this also must not be able to bind in a β-strand conformation. Moreover the corresponding alcohols (**3.72** and **3.75)** possess negligible calpain inhibition thus further supporting the hypothesis that neither of these types of compounds are able to exist in a β-strand conformation.

3.7: Conclusions and future work

The synthesis of an eight membered cyclic nitrogen to nitrogen analogue of SJA-6017 (**2.12**) was attempted. Cyclisation was achieved by allylating both nitrogen protons of a suitably protected **2.12** analogue and performing RCM on this diene. However, the cyclic methyl esters **3.33** and **3.39** were unexpectedly very sensitive to reducing reagents and as such the synthesis of a *N*-*N* cyclised calpain inhibitor was not possible.

Synthetic methodology was developed to allow the facile synthesis of the nine and ten membered ring analogues of cyclic methyl esters **3.33** and **3.39**. This could be worth pursuing with the objective of preparing a publication outlining the use of a sulfonamide as an approach

to achieve selective allylation and thus methodology to prepare different ring sized *N*-*N* cyclised dipeptides.

The synthesis of conformationally constrained β-amino acid calpain inhibitor **3.73** was successfully achieved. This proved to be a moderate calpain inhibitor with an IC_{50} of 3.15 μM. It is equipotent to its direct acyclic analogue (**3.76**) which was also successfully prepared.

References for Chapter 3

1. Galande, A. K.; Trent, J. O.; Spatola, A. F., Understanding base-assisted desulfurization using a variety of disulfide-bridged peptides. *Biopolymers* **2003,** 71, (5), 534-551.

2. Reichwein, J. F.; Wels, B.; Kruijtzer, J. A. W.; Versluis, C.; Liskamp, R. M. J., Rolling loop scan: An approach featuring ring-closing metathesis for generating libraries of peptides with molecular shapes mimicking bioactive conformations or local folding of peptides and proteins. *Angewandte Chemie-International Edition* **1999,** 38, (24), 3684-3687.

3. Cowell, S. M.; Lee, Y. S.; Cain, J. P.; Hruby, V. J., Exploring Ramachandran and Chi space: conformationally constrained amino acids and peptides in the design of bioactive polypeptide ligands. *Current Medicinal Chemistry* **2004,** 11, (21), 2785-2798.

4. Anderson, A. W.; Merckling, N. G. Polymeric bicyclo[2.2.1]hept-2-ene. 2721189, 1955.

5. Calderon, N.; Chen, H. Y.; Scott, K. W., Olefin Metathesis - a Noval Reaction for Skeletal Transformations of Unsaturated Hydrocarbons. *Tetrahedron Letters* **1967**, (34), 3327-&.

6. Herisson, J. L.; Chauvin, Y., Transformation Catalysis of Olefins by Tungsten Complexes .2. Telomerization of Cyclic Olefins in Presence of Acyclic Olefins. *Makromolekulare Chemie* **1971,** 141, (FEB9), 161-&.

7. Leconte, M. B., J. M.; Quignard, F.; Larroche, C, *Mechanistic Aspects of the Olefin Metathesis Reaction. In Reactions of Coordinated Ligands*. Plenum: New York, 1986; Vol. 1 p371-420.

8. Grubbs, R. H., *Alkene and Alkyne Metathesis Reactions*. Pergamon: Oxford, 1982; Vol. 8.

9. Katz, T. J.; McGinnis, J., Metathesis of Cyclic and Acyclic Olefins. *Journal of the American Chemical Society* **1977,** 99, (6), 1903-1912.

10. Grubbs, R. H.; Carr, D. D.; Hoppin, C.; Burk, P. L., Consideration of Mechanism of Metal-Catalyzed Olefin Metathesis Reaction. *Journal of the American Chemical Society* **1976,** 98, (12), 3478-3483.

11. Katz, T. J.; Rothchild, R., Mechanism of Olefin Metathesis of 2,2'-Divinylbiphenyl. *Journal of the American Chemical Society* **1976,** 98, (9), 2519-2526.

12. Katz, T. J.; McGinnis, J., Mechanism of Olefin Metathesis Reaction. *Journal of the American Chemical Society* **1975,** 97, (6), 1592-1594.

13. Grubbs, R. H.; Burk, P. L.; Carr, D. D., Consideration of Mechanism of Olefin Metathesis Reaction. *Journal of the American Chemical Society* **1975,** 97, (11), 3265-3267.

14. Schrock, R.; Rocklage, S.; Wengrovius, J.; Rupprecht, G.; Fellmann, J., Preparation and Characterization of Active Niobium, Tantalum and Tungsten Metathesis Catalysts. *Journal of Molecular Catalysis* **1980,** 8, (1-3), 73-83.

15. Wengrovius, J. H.; Schrock, R. R.; Churchill, M. R.; Missert, J. R.; Youngs, W. J., Multiple Metal-Carbon Bonds .16. Tungsten-Oxo Alkylidene Complexes as Olefin Metathesis Catalysts and the Crystal-Structure of W(O)(Chcme3)(Pet3)Cl2. *Journal of the American Chemical Society* **1980,** 102, (13), 4515-4516.

16. Kress, J. R. M.; Osborn, J. A.; Wesolek, M. G. Oxo complexes of transition metals coordinated with a Lewis acid their use as catalysts in the metathesis of olefins. 81-2293 2499083, 19810204., 1982.

17. Katz, T. J.; Sivavec, T. M., Metal-Catalyzed Rearrangement of Alkene Alkynes and the Stereochemistry of Metallacyclobutene Ring-Opening. *Journal of the American Chemical Society* **1985,** 107, (3), 737-738.

18. Katz, T. J.; Lee, S. J.; Acton, N., Stereospecific Polymerizations of Cycloalkenes Induced by a Metal-Carbene. *Tetrahedron Letters* **1976**, (47), 4247-4250.

19. Grubbs, R. H.; Tumas, W., Polymer Synthesis and Organotransition Metal Chemistry. *Science* **1989,** 243, (4893), 907-915.

20. Wallace, K. C.; Liu, A. H.; Dewan, J. C.; Schrock, R. R., Preparation and Reactions of Tantalum Alkylidene Complexes Containing Bulky Phenoxide or Thiolate Ligands - Controlling Ring-Opening Metathesis Polymerization Activity and Mechanism through Choice of Anionic Ligand. *Journal of the American Chemical Society* **1988,** 110, (15), 4964-4977.

21. Kress, J.; Osborn, J. A.; Greene, R. M. E.; Ivin, K. J.; Rooney, J. J., 1st Direct Observation of the Simultaneous Presence and of the Interconversion of Chain-Propagating Metal-Carbene and Metallacyclobutane Complexes in a Catalytic Olefin Metathesis Reaction - the Ring-Opening Polymerization of Norbornene. *Journal of the American Chemical Society* **1987,** 109, (3), 899-901.

22. Kress, J.; Aguero, A.; Osborn, J. A., Recent Advances in the Chemistry of Tungsten-Carbene Complexes. *Journal of Molecular Catalysis* **1986,** 36, (1-2), 1-12.

23. Quignard, F.; Leconte, M.; Basset, J. M., Synthesis and Catalytic Properties of W(Oar)2cl2(Chcme3)(Or2), and W(Oar)2cl(Chcme3)(Ch2cme3)(Or2) (Ar = 2,6-Disubstituted Phenyl, R = Et or Pri), New Uni-Component Catalysts for Metathesis of Acyclic

and Cyclic Olefins, with or without Functional-Groups. *Journal of the Chemical Society-Chemical Communications* **1985**, (24), 1816-1817.

24. Schrock, R. R.; Murdzek, J. S.; Bazan, G. C.; Robbins, J.; Dimare, M.; Oregan, M., Synthesis of Molybdenum Imido Alkylidene Complexes and Some Reactions Involving Acyclic Olefins. *Journal of the American Chemical Society* **1990,** 112, (10), 3875-3886.

25. Oskam, J. H.; Fox, H. H.; Yap, K. B.; McConville, D. H.; Odell, R.; Lichtenstein, B. J.; Schrock, R. R., Ligand Variation in Alkylidene Complexes of the Type Mo(Chr)(Nr')(or")2. *Journal of Organometallic Chemistry* **1993,** 459, (1-2), 185-198.

26. Feldman, J.; Murdzek, J. S.; Davis, W. M.; Schrock, R. R., Reaction of Neopentylidene Complexes of the Type M(Ch-T-Bu)(N-2,6-C6h3-I-Pr2)(or)2 (M = W, Mo) with Methyl Acrylate and N,N-Dimethylacrylamide to Give Metallacyclobutane Complexes. *Organometallics* **1989,** 8, (9), 2260-2265.

27. Kirkland, T. A.; Grubbs, R. H., Effects of olefin substitution on the ring-closing metathesis of dienes. *Journal of Organic Chemistry* **1997,** 62, (21), 7310-7318.

28. Shon, Y. S.; Lee, T. R., Catalytic ring-closing olefin metathesis of sulfur-containing species: Heteroatom and other effects. *Tetrahedron Letters* **1997,** 38, (8), 1283-1286.

29. Armstrong, S. K.; Christie, B. A., Synthesis of sulphur-containing heterocycles by ring closing diene metathesis. *Tetrahedron Letters* **1996,** 37, (52), 9373-9376.

30. Armstrong, S. K., Ring closing diene metathesis in organic synthesis. *Journal of the Chemical Society-Perkin Transactions 1* **1998**, (2), 371-388.

31. Bujard, M.; Gouverneur, V.; Mioskowski, C., A highly efficient and practical synthesis of cyclic phosphinates using ring-closing metathesis. *Journal of Organic Chemistry* **1999,** 64, (6), 2119-2123.

32. Barrett, A. G. M.; Baugh, S. P. D.; Braddock, D. C.; Flack, K.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; Procopiou, P. A.; White, A. J. P.; Williams, D. J., Rapid entry into mono-, bi-, and tricyclic beta-lactam arrays via alkene metathesis. *Journal of Organic Chemistry* **1998,** 63, (22), 7893-7907.

33. Fujimura, O.; Fu, G. C.; Grubbs, R. H., The Synthesis of Cyclic Enol Ethers Via Molybdenum Alkylidene-Catalyzed Ring-Closing Metathesis. *Journal of Organic Chemistry* **1994,** 59, (15), 4029-4031.

34. Clark, J. S.; Kettle, J. G., Synthesis of brevetoxin sub-units by sequential ring-closing metathesis and hydroboration. *Tetrahedron Letters* **1997,** 38, (1), 123-126.

35. Clark, J. S.; Kettle, J. G., Enantioselective synthesis of medium-ring sub-units of brevetoxin A by ring-closing metathesis. *Tetrahedron Letters* **1997,** 38, (1), 127-130.

36. Calimente, D.; Postema, M. H. D., Preparation of C-1 glycals via olefin metathesis. A convergent and flexible approach to C-glycoside synthesis. *Journal of Organic Chemistry* **1999,** 64, (6), 1770-1771.

37. Postema, M. H. D.; Calimente, D., Convergent preparation of 1,6-linked C-disaccharides via olefin metathesis. *Tetrahedron Letters* **1999,** 40, (26), 4755-4759.

38. Hodgson, D. M.; Foley, A. M.; Lovell, P. J., Alkene chemoselectivity in ring-closing metathesis: A formal synthesis of (-)-periplanone-B. *Synlett* **1999**, (6), 744-746.

39. Sturino, C. F.; Wong, J. C. Y., The ring-closing metathesis of vinyl ethers with Grubbs' catalyst for the synthesis of dihydropyrans. *Tetrahedron Letters* **1998,** 39, (52), 9623-9626.

40. Grubbs, R. H., The Development of Functional-Group Tolerant Romp Catalysts. *Journal of Macromolecular Science-Pure and Applied Chemistry* **1994,** A31, (11), 1829-1833.

41. Michelot.Fw; Keaveney, W. P., Coordinated Polymerization of Bicyclo-[2.2.1]-Heptene-2 Ring System (Norbornene) in Polar Media. *Journal of Polymer Science Part a-General Papers* **1965,** 3, (3PA), 895-&.

42. Rinehart, R. E.; Smith, H. P., Emulsion Polymerization of Norbornene Ring System Catalyzed by Noble Metal Compounds. *Journal of Polymer Science Part B-Polymer Letters* **1965,** 3, (12PB), 1049-&.

43. Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H.; Ziller, J. W., Ring-Opening Metathesis Polymerization (Romp) of Norbornene by a Group-Viii Carbene Complex in Protic Media. *Journal of the American Chemical Society* **1992,** 114, (10), 3974-3975.

44. Schrock, R. R., Olefin metathesis by molybdenum imido alkylidene catalysts. *Tetrahedron* **1999,** 55, (27), 8141-8153.

45. Schrock, R. R., Living Ring-Opening Metathesis Polymerization Catalyzed by Well-Characterized Transition-Metal Alkylidene Complexes. *Accounts of Chemical Research* **1990,** 23, (5), 158-165.

46. Fu, G. C.; Nguyen, S. T.; Grubbs, R. H., Catalytic Ring-Closing Metathesis of Functionalized Dienes by a Ruthenium Carbene Complex. *Journal of the American Chemical Society* **1993,** 115, (21), 9856-9857.

47. Bourissou, D.; Guerret, O.; Gabbai, F. P.; Bertrand, G., Stable carbenes. *Chemical Reviews* **2000,** 100, (1), 39-91.

48. Weskamp, T.; Schattenmann, W. C.; Spiegler, M.; Herrmann, W. A., A novel class of ruthenium catalysts for olefin metathesis. *Angewandte Chemie-International Edition* **1998,** 37, (18), 2490-2493.

49. Huang, J. K.; Stevens, E. D.; Nolan, S. P.; Petersen, J. L., Olefin metathesis-active ruthenium complexes bearing a nucleophilic carbene ligand. *Journal of the American Chemical Society* **1999,** 121, (12), 2674-2678.

50. Huang, J. K.; Schanz, H. J.; Stevens, E. D.; Nolan, S. P., Influence of sterically demanding carbene ligation on catalytic behavior and thermal stability of ruthenium olefin metathesis catalysts. *Organometallics* **1999,** 18, (25), 5375-5380.

51. Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H., Increased ring closing metathesis activity of ruthenium-based olefin metathesis catalysts coordinated with imidazolin-2-ylidene ligands. *Tetrahedron Letters* **1999,** 40, (12), 2247-2250.

52. Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H., Synthesis and activity of a new generation of ruthenium-based olefin metathesis catalysts coordinated with 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene ligands. *Organic Letters* **1999,** 1, (6), 953-956.

53. Chatterjee, A. K.; Grubbs, R. H., Synthesis of trisubstituted alkenes via olefin cross-metathesis. *Organic Letters* **1999,** 1, (11), 1751-1753.

54. Chatterjee, A. K.; Morgan, J. P.; Scholl, M.; Grubbs, R. H., Synthesis of functionalized olefins by cross and ring-closing metatheses. *Journal of the American Chemical Society* **2000,** 122, (15), 3783-3784.

55. Ackermann, L.; Furstner, A.; Weskamp, T.; Kohl, F. J.; Herrmann, W. A., Ruthenium carbene complexes with imidazolin-2-ylidene ligands allow the formation of tetrasubstituted cycloalkenes by RCM. *Tetrahedron Letters* **1999,** 40, (26), 4787-4790.

56. Weskamp, T.; Kohl, F. J.; Hieringer, W.; Gleich, D.; Herrmann, W. A., Highly active ruthenium catalysts for olefin metathesis: The synergy of N-heterocyclic carbenes and coordinatively labile ligands. *Angewandte Chemie-International Edition* **1999,** 38, (16), 2416-2419.

57. Weskamp, T.; Kohl, F. J.; Herrmann, W. A., N-heterocyclic carbenes: novel ruthenium-alkylidene complexes. *Journal of Organometallic Chemistry* **1999,** 582, (2), 362-365.

58. Furstner, A.; Thiel, O. R.; Ackermann, L.; Schanz, H. J.; Nolan, S. P., Ruthenium carbene complexes with N,N '-bis(mesityl) imidazol-2-ylidene ligands: RCM catalysts of extended scope. *Journal of Organic Chemistry* **2000,** 65, (7), 2204-2207.

59. Miller, S. J.; Blackwell, H. E.; Grubbs, R. H., Application of ring-closing metathesis to the synthesis of rigidified amino acids and peptides. *Journal of the American Chemical Society* **1996,** 118, (40), 9606-9614.

60. Reichwein, J. F.; Liskamp, R. M. J., Synthesis of cyclic dipeptides by ring-closing metathesis. *European Journal of Organic Chemistry* **2000**, (12), 2335-2344.

61. Reichwein, J. F.; Versluis, C.; Liskamp, R. M., Synthesis of cyclic peptides by ring-closing metathesis. *The Journal of organic chemistry* **2000,** 65, (19), 6187-95.

62. Reichwein, J. F.; Versluis, C.; Liskamp, R. M. J., Synthesis of cyclic peptides by ring-closing metathesis. *Journal of Organic Chemistry* **2000,** 65, (19), 6187-6195.

63. Bihovsky, R.; Tao, M.; Mallamo, J. P.; Wells, G. J., 1,2-Benzothiazine 1,1-dioxide alpha-ketoamide analogues as potent calpain I inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2004,** 14, (4), 1035-1038.

64. Payne, R. J.; Brown, K. M.; Coxon, J. M.; Morton, J. D.; Lee, H. Y.-Y.; Abell, A. D., Peptidic Aldehydes Based on a- and b-Amino Acids: Synthesis, Inhibition of m-Calpain, and Anti-Cataract Properties. *Australian Journal of Chemistry* **2004,** 57, (9), 877-884.

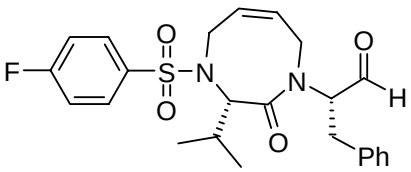
65. Huang, L.; Lee, A.; Ellman, J. A., Identification of potent and selective mechanism-based inhibitors of the cysteine protease cruzain using solid-phase parallel synthesis. *Journal of Medicinal Chemistry* **2002,** 45, (3), 676-684.

66. Nakamura, M.; Yamaguchi, M.; Sakai, O.; Inoue, J., Exploration of cornea permeable calpain inhibitors as anticataract agents. *Bioorganic & Medicinal Chemistry* **2003,** 11, (7), 1371-1379.

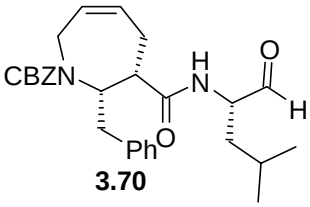
67. Matthews, J. L.; Braun, C.; Guibourdenche, C.; Overhand, M.; Seebach, D., Preparation of enantiopure b-amino acids from a-amino acids using the Arndt-Eistert homologation. *Enantioselective Synthesis of b-Amino Acids* **1997**, 105-126.

4.1: Use of macrocyclisation to constraint a peptidomimetic in a β-strand conformation

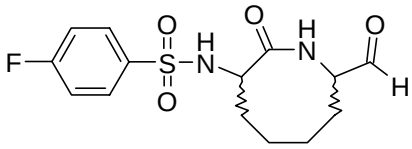
Chapter 2 described the successful design and synthesis of acyclic compounds which molecular modeling showed were able to adopt a β-strand conformation. **Chapter 3** described the use of RCM to synthesise cyclic dipeptides with the objective of conformationally constraining these into a β-strand conformation. However, the carbon to nitrogen conformationally constrained dipeptide (**3.70**) was a poor calpain inhibitor while molecular modeling suggested **3.35** was a poor β-strand mimetic. Furthermore previous work in our laboratory, where a carbon to carbon conformational constraint[1] as in **4.0** was synthesised, proved to be a very poor calpain inhibitor. As such it was concluded that these were not β-strand mimics.



3.35

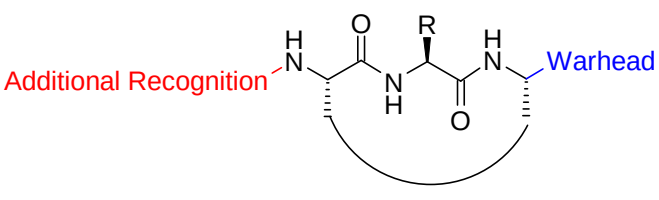


3.70



4.0

The cyclic compounds described above (**3.35**, **3.70** and **4.0**) were designed on the basis of the generic calpain inhibitor template (**2.7**), see **Section 2.1**. These findings provided very clear evidence that if a successful cyclic β-strand mimic was to be designed using a conformational constraint strategy, then more was required than simply constructing conformational constraints of generic dipeptide motif **2.7**. This chapter describes the design and implementation of a carbon to carbon macrocyclisation strategy to conformationally constrain tripeptides into a β-strand conformation. As such a new generic structure was adopted as the design template (**4.1**).



4.1

There were two main reasons why this new template was chosen.

- As the conformational constraint is incorporated as a carbon to carbon backbone cyclisation, the template is capable of forming the three key β-strand hydrogen bonds to the protease (to Gly_{208} and Gly_{271} in the case of calpain). See **Section 2.1**.

- The central amino acid (R) in **4.1** ensures that it is still capable of forming the complementary P2/S2 enzyme/substrate interaction.

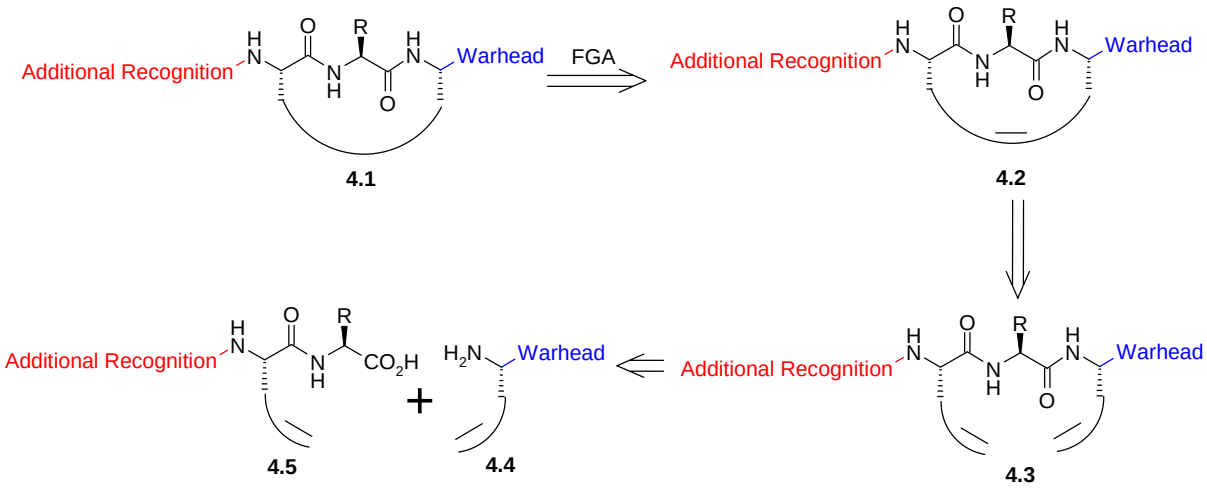
Macrocyclisation was also an attractive strategy for two other reasons;

- Macrocyclic compounds with amide bonds have been demonstrated to possess higher resistance to proteolytic cleavage relative to their acyclic analogues.[2]

- Macrocyclic compounds often exhibit improved cell permeability compared to their acyclic counterparts.[3, 4]

4.2: Generation of an *in-silico* library of macrocyclic β-strand templates

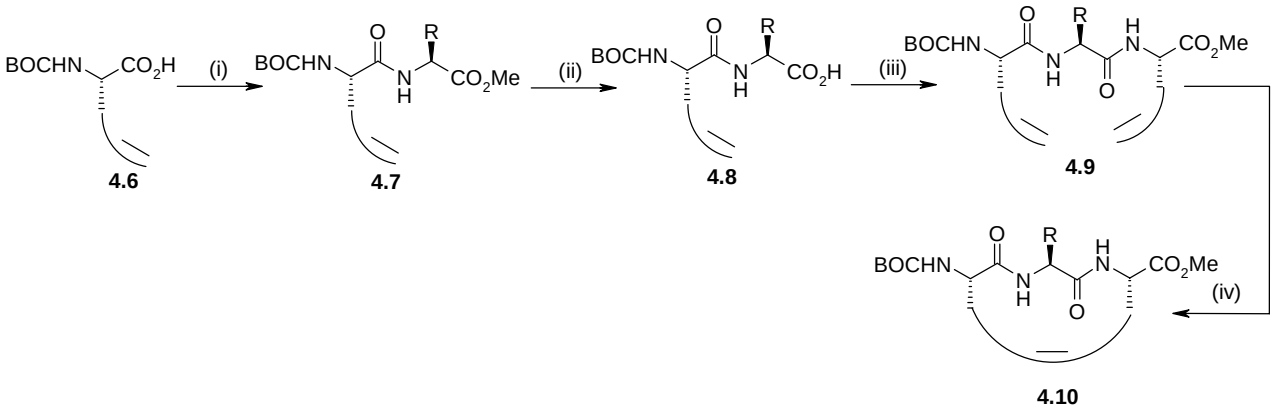
A retrosynthetic analysis of macrocyclic β-strand template **4.1** using RCM the key cyclisation step was performed (**Scheme 4.1)**. To use RCM chemistry **4.1** must be disconnected to a diene precursor (**4.3**), which in turn is disconnected to two side chain derivatised amino acids (**4.4** and **4.5**).



Scheme 4.1 Retrosynthetic analysis of **4.1**

Based on this general retrosynthetic scheme it was necessary to employ a protecting group strategy. A key requirement of the synthetic route was the ability to vary the *N*-substituent of the macrocycle easily. An advanced intermediate, with an easily cleaved amine protecting group, was required to minimise the number of synthetic steps. Furthermore, an aldehyde was chosen as the warhead for these initial studies. The strategy was to determine the optimum β-strand macrocyclic mimic using an aldehyde and to subsequently append different warheads.

A generic synthesis of the macrocyclic templates is shown in **Scheme 4.2**. A BOC protected amino acid with an olefin in the side chain (**4.6**) is coupled with an amino acid methyl ester to give a dipeptide (**4.7**). This is hydrolysed to carboxylic acid **4.8** and coupled with a second amino acid containing an olefin in the side chain with the C-terminus protected as a methyl ester to give diene **4.9**. RCM of this affords orthogonally protected macrocycle **4.10**.



Scheme 4.2. *Reagents and Conditions:* **(i)** HATU, DIPEA, H_2N-AA-OMe, DMF; **(ii)** NaOH, THF, H_2O, MeOH; **(iii)** HATU, DIPEA, H_2N-AA(allyl)-OMe, DMF; **(iv) 3.9**, solvent.

With this proposed general synthetic route in hand an *in-silico* combinatorial library of potential macrocycles was prepared. This was composed of an 18 X 16 array of derivatised amino acids synthesised with either N or C protection containing an allyl group in the side chain. An excel spreadsheet was constructed containing all possible macrocyclic compounds which could be prepared from the eighteen *N*-BOC-allyl amino acids and the sixteen allyl

amino acid methyl esters (**Figure 4.3**) using combinatorial chemistry methodology. Sections of the excel spreadsheet are shown in **Figures 4.1 and 4.2**.

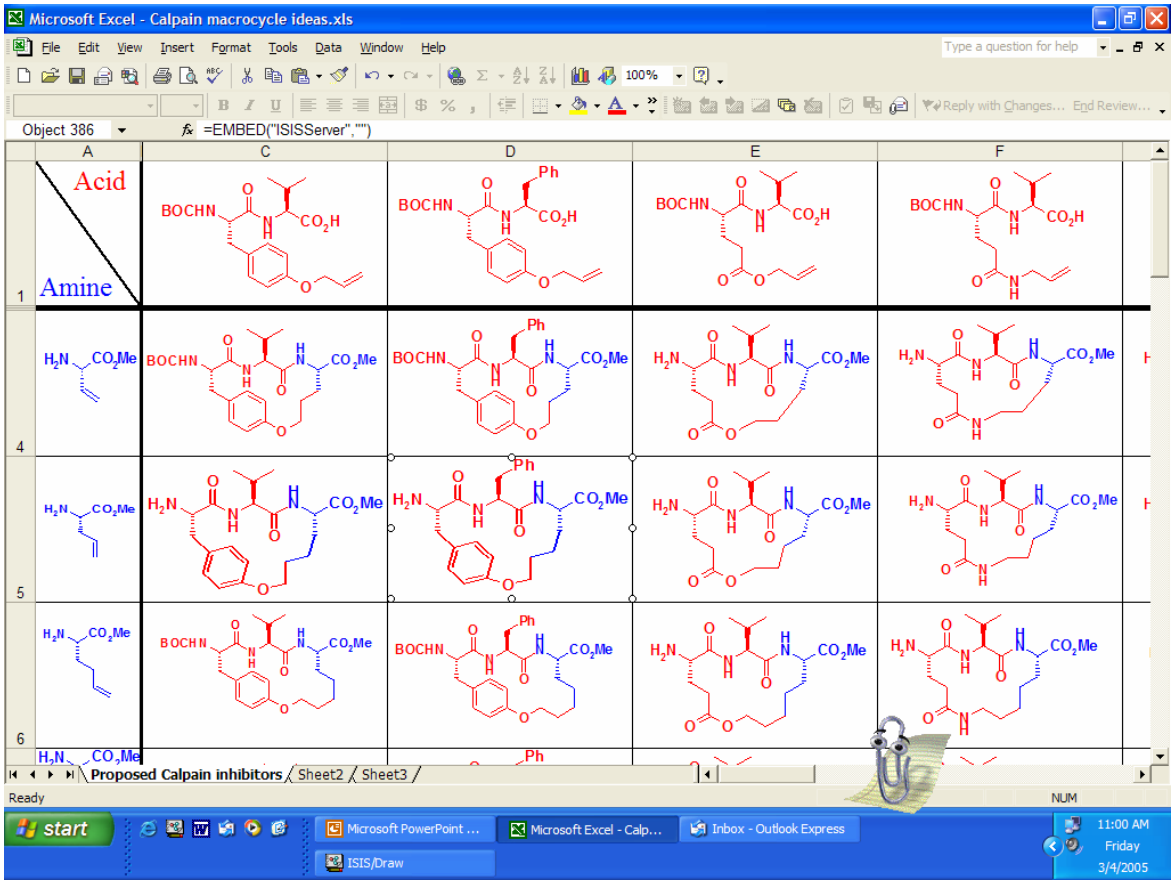


Figure 4.1: *In-silico* β-strand combinatorial library

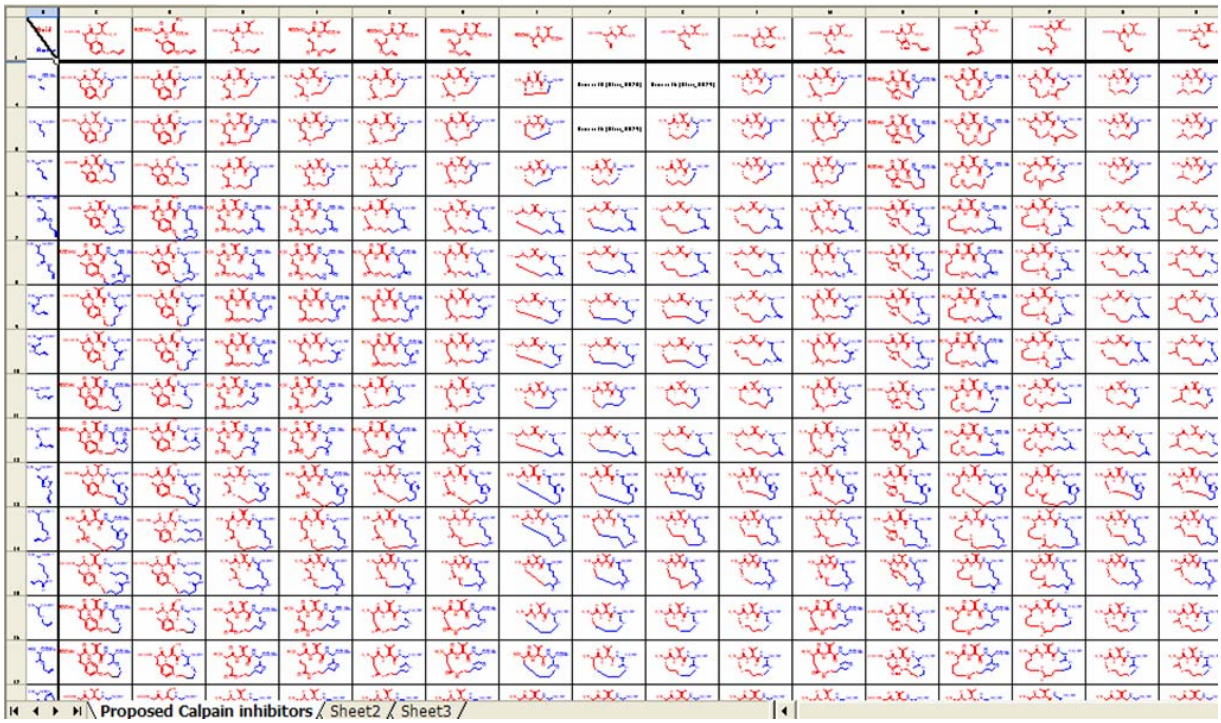


Figure 4.2: Virtual library of a 18 X 16 array (288 compounds)

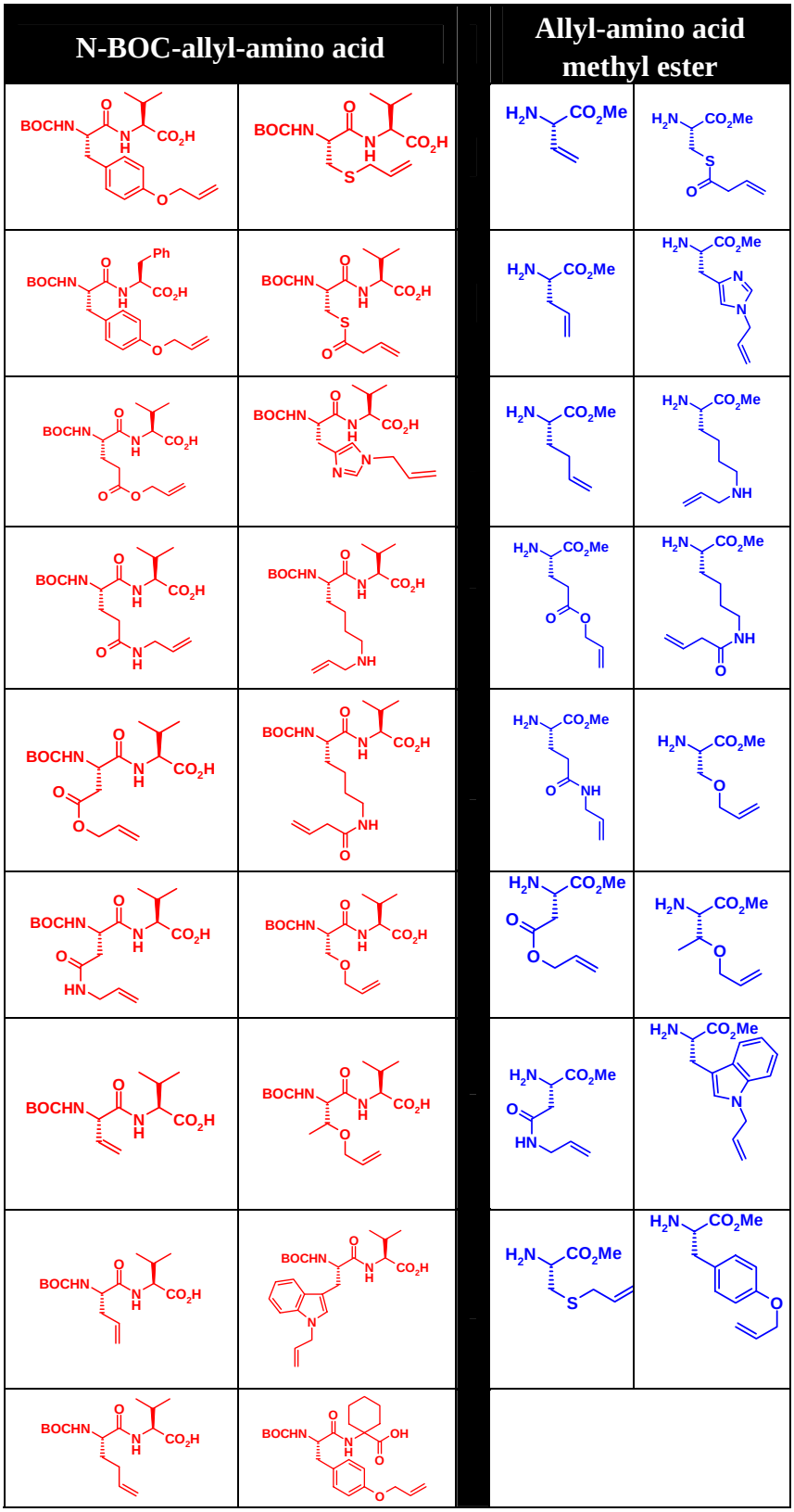


Figure 4.3: Side chain allylated amino acids composing the *in-silico* library

4.3: Conformational analysis of the *in-silico* library

Boltzmann weighted conformational analysis was used to determine if a particular macrocycle could adopt the β-strand conformation. Conformational searches were conducted using the Monte Carlo multiple minimum (MCMM) method. These were run with a generalised Born/surface area (GB/SA) water model using the OPLS2001 force field. Settings of 1000 steps for the conformational search and up to 500 iterations for the minimisation of each generated structure were used. The default Polak-Ribiere conjugate gradient method was used for all minimisations and this was stopped when the default gradient convergence threshold of D = 0.05 kJ/(mol*Å) was reached.

The conformation of a specific conformer was determined using a numerical method to assign this as either β-strand, β-twist or β-turn. As shown in **Figure 4.4** the distance between the NH of the first amino acid and the carbonyl of the central amino acid was used.

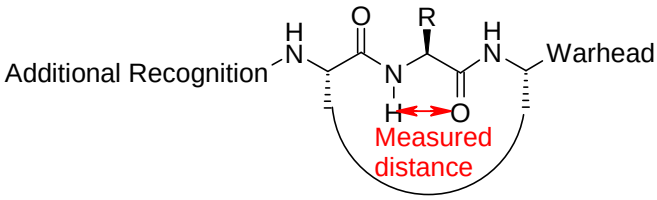


Figure 4.4: Measurement used to define a β-strand or β-turn conformation

An optimal β-strand had a distance of approximately 2.5Å and a clear β-turn occurred when the distance was greater than 3.7Å. As such the definitions used in this thesis are;

- β-strand is < 3.1Å
- β-twist (an intermediate case between β-strand and β-turn conformation) is 3.11 to 3.69Å.
- β-turn conformation >3.7Å.

An example of the implementation of this methodology is shown in **Figure 4.4b**.

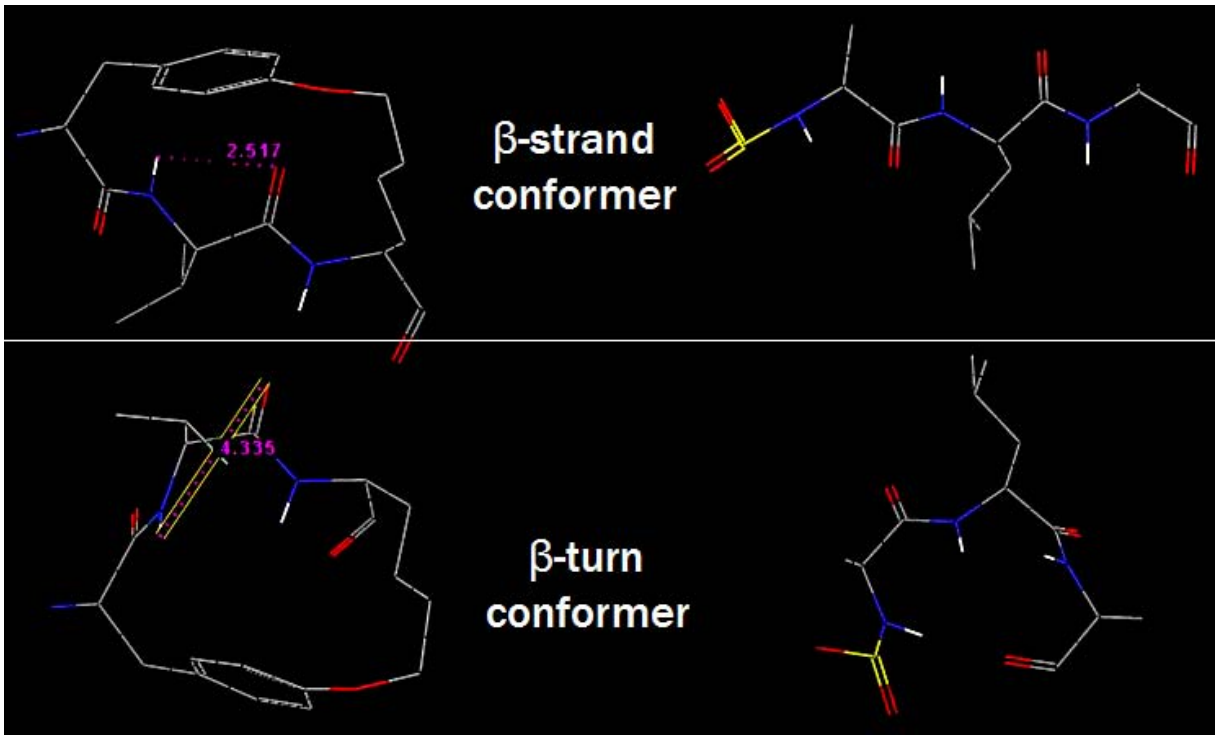


Figure 4.4b: Distance measured in order to assign a structure as either a β-strand or β-turn

An ensemble of low energy conformers for each structure was generated using a 12kJ/mol/Å window. A Boltzmann weighted conformational analysis was then performed on this ensemble, and a weighted percentage of the three different conformations was calculated. An example of this is shown in **Table 4.1** with the three far right columns (orange highlighted) indicating the percentage of each conformation for a particular macrocycle;

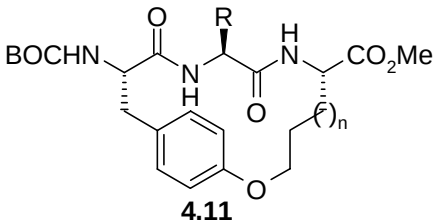
Energy	E/kT	e-E/kT	Ne-E/kT	Boltzmann	%	E* %	Distance 24-50	Bolt.Ave.E	Strand	Turn	Twist %	Strand %	Turn %
-553.31	221.83	###	###	12.19	33.87	-18738.59	2.49	-549.61	33.87	0	23.95	76.05	0
-551	220.9	###	###	4.83	13.41	-7388.08	2.18		13.41	0			
-550.5	220.7	###	###	3.95	10.96	-6033.06	2.13		10.96	0			
-548.51	219.9	###	###	1.78	4.94	-2710.91	2.78		4.94	0			
-546.72	219.18	###	###	0.87	2.41	-1319.58	3.64		0	0			
-546.72	219.18	###	###	0.87	2.41	-1315.66	3.64		0	0			
-546.71	219.18	###	###	0.87	2.4	-1314.14	3.64		0	0			
-546.71	219.18	###	###	0.86	2.4	-1311.82	3.64		0	0			
-546.71	219.18	###	###	0.86	2.4	-1310.59	3.64		0	0			
-546.7	219.17	###	###	0.86	2.39	-1307.31	3.64		0	0			
-546.69	219.17	###	###	0.86	2.38	-1301.38	3.64		0	0			
-546.68	219.17	###	###	0.85	2.37	-1297.74	3.64		0	0			
-546.68	219.17	###	###	0.85	2.37	-1296.05	3.64		0	0			
-546.02	218.9	###	###	0.66	1.82	-993.47	2.14		1.82	0			
-545.93	218.87	###	###	0.63	1.75	-957.56	3.51		0	0			
-545.51	218.7	###	###	0.53	1.48	-808.17	2.14		1.48	0			
-544.97	218.48	###	###	0.43	1.2	-651.46	2.2		1.2	0			
-544.61	218.34	###	###	0.37	1.03	-563.14	2.17		1.03	0			
-544.38	218.24	###	###	0.34	0.94	-512.6	2.07		0.94	0			
-543.89	218.05	###	###	0.28	0.78	-422.25	2.8		0.78	0			
-543.43	217.87	###	###	0.23	0.65	-350.66	2.2		0.65	0			
-543.22	217.78	###	###	0.21	0.59	-321.51	2.82		0.59	0			
-542.42	217.46	###	###	0.15	0.43	-233.11	2.71		0.43	0			
-542.3	217.41	###	###	0.15	0.41	-222.35	2.13		0.41	0			
-542.27	217.4	###	###	0.15	0.41	-219.82	2.71		0.41	0			

Table 4.1: Conformational analysis using Boltzmann weighted distribution

This analysis generated a number of macrocyclic structures which appeared to be moderate to excellent β-strand mimics. The best of these, as discussed below, were selected for synthesis.

4.4: Synthesis of 17-membered Tyr-aa-Gly based macrocycles

As described in **Sections 4.2** and **4.3** conformational analysis was used to prioritise the macrocycles for synthesis. The prototype macrocyclic system targeted to validate the retrosynthetic strategy in **Scheme 4.2** was the Tyr-aa-Gly system of variable ring size (n = 1 to 3) (**4.11).**



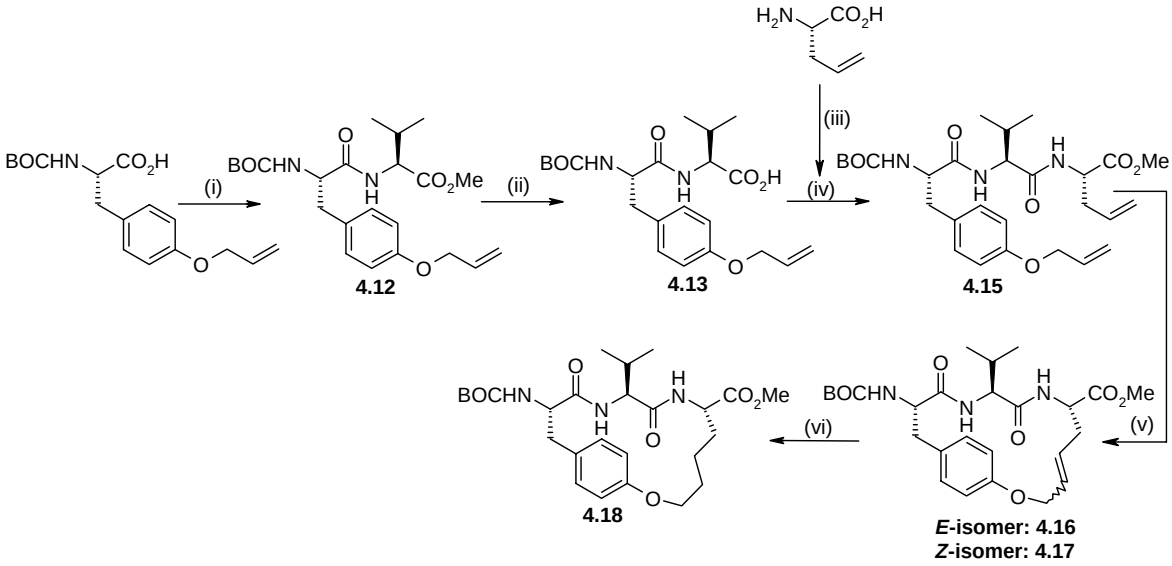
4.11

These were attractive targets primarily for two reasons;

- The ring size could be easily varied by using the appropriate C-allylated amino acid, namely, (S)-vinyl-Gly-OMe, (S)-allyl-Gly-OMe or (S)-homo-allyl-Gly-OMe,
- All three ring sizes (16-18) were predicted by molecular modelling to exist predominantly as a β-strand.

The synthesis of the seventeen membered ring macrocycle is shown in **Scheme 4.3.** Commercial *N*-BOC-O-allyl-Tyr-H was coupled with Val-OMe using standard HATU peptide coupling conditions to yield dipeptide **4.12**. The ester was hydrolysed with sodium hydroxide and resultant carboxylic acid **4.13** was coupled with (S)-allyl-Gly-OMe to afford diene **4.15**. The (S)-allyl-Gly-OMe (**4.14**) was obtained from an acid catalysed esterification reaction of (S)-allyl-Gly based on a published literature procedure.[5] RCM was performed under a variety of conditions (discussed in **Section 4.5**) to prepare the ring closed product as a mixture of geometric isomers (**4.16** and **4.17**, discussed in **Section 4.6**). Hydrogenation at

atmospheric pressure and room temperature, with palladium on carbon, afforded orthogonally protected macrocycle **4.18** in quantitative yield.



Scheme 4.3. *Reagents and Conditions:* **(i)** HATU, DIPEA, Val-OMe, DMF, (86%); **(ii)** NaOH, THF, H_2O, MeOH, (97%); **(iii)** $SOCl_2$, MeOH, (100%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe (**4.14**), DMF, (83%); **(v)** 10 mol% **3.9**, DCM, rt, (0%); or 10 mol% **3.9**, DCM, reflux, (0%); or 3 x 10 mol% **3.9**, 1,1,2-TCE, reflux, (12%, **4.16** and 31%, **4.17**); or 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (68%, **4.17**); **(vi)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (100%)

4.5: Microwave assisted RCM

RCM reactions are generally carried out at room temperature or slightly elevated temperatures (e.g. refluxing dichloromethane). However, in cases where cyclisation does not proceed moderately elevated temperatures are often required. The macrocyclisation of diene **4.15** required an elevated temperature (refluxing 1,1,2-trichloroethane) for several hours to obtain its maximum yield. However, it was postulated that the use of microwave irradiation might prove useful to accelerate the reaction rate since Grubbs second generation catalyst decomposes at elevated temperature.

Since its widespread introduction during the late 1990s the use of microwave irradiation as a non-conventional energy source has been applied to numerous chemical reactions.[6-8]

Microwave heating has also been demonstrated to expedite metal-catalysed organic synthesis. Palladium catalysed transformations are the most extensively studied,[9-11] but molybdenum,[12, 13] and very recently ruthenium[14-16] have also been the focus of research. The use of microwave irradiation in the field of RCM is very much in its infancy. This was the subject of a review published by our laboratory in 2005.[17] To the best of our knowledge this was the first review of microwave irradiation in the field of RCM chemistry.

With this knowledge in hand microwave irradiation to promote RCM appeared to hold significant potential. This was applied to the synthesis of our target macrocycles (**Scheme 4.3**). The use of microwave irradiation in the RCM reaction of diene **4.15** resulted in both an increased yield (50% increase relative to the thermal reaction) and a shorter reaction time (one hour instead of the eighteen hours for thermal reflux).

The reason for this reaction rate acceleration, and improved yield, is postulated to be primarily due to the fact that microwave heating transfers heat directly into the reaction, rather than via the vessel walls. A thermally unstable such as Grubbs second generation catalyst would be expected to be more stable in the reaction media relative to the hot vessel walls. This is in agreement with previous studies which have demonstrated that the use of microwave irradiation in the area of RCM results in both an increase in reaction rate and a decrease in the rate of catalyst decomposition.[17]

The microwave irradiation reaction conditions developed in **Scheme 4.3** were adopted as the optimal conditions for all subsequent macrocyclic RCM reactions discussed in this thesis. A further extension of these conditions was later developed whereby 10 mol% of a Lewis acid (chloro-dicyclohexyl borane) was used as an additive to increase the yield of RCM reactions (see **Section 4.19**).

4.6: Effect of microwave irradiation on the *E/Z* isomer ratios

As described throughout this Chapter, macrocyclisation using RCM results in an *E/Z* mixture of product macrocycles. The geometric isomers **4.16** and **4.17** obtained in **Scheme 4.3** were separated and the configuration of each geometric isomer assigned on the basis of nOe. The key difference between the two isomers was that an nOe was observed between the α-Gly proton and one of methylene protons adjacent to the tyrosine oxygen in only one isomer. In the *Z*-isomer the Boltzmann weighted distance between these two protons was calculated, using Macromodel 9.0, to be 4.9Å, and in the *E*-isomer it was calculated to be 6.1Å (**Figure 4.5)**. Accordingly the assignments were made on this basis.

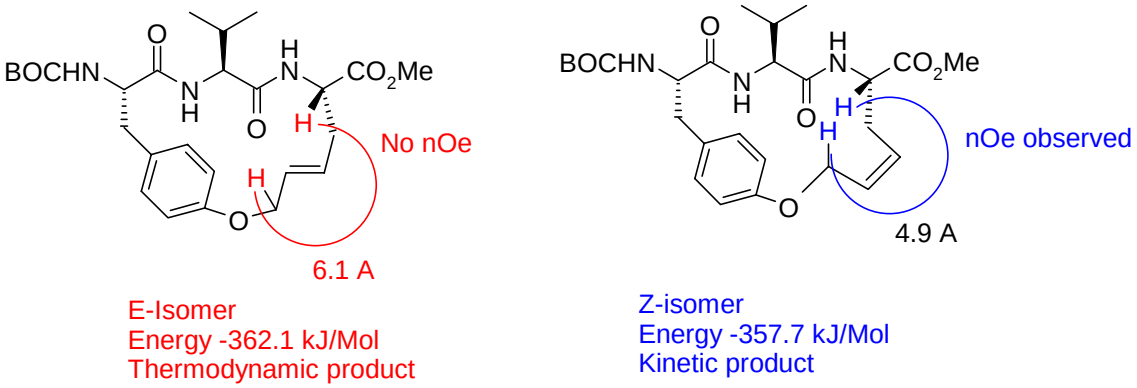


Figure 4.5: Assignment of geometric isomers from **Scheme 4.3**

A summary of the ratio of geometric isomers obtained in the cyclisation of **4.15** under both thermal and microwave heating is shown in **Table 5.2.**

Heating method	Yield (%)	E:Z ratio
Thermal	43	2.5:1
Microwave	68	0:100

Table 5.2: Geometric isomer ratios using thermal and microwave heating

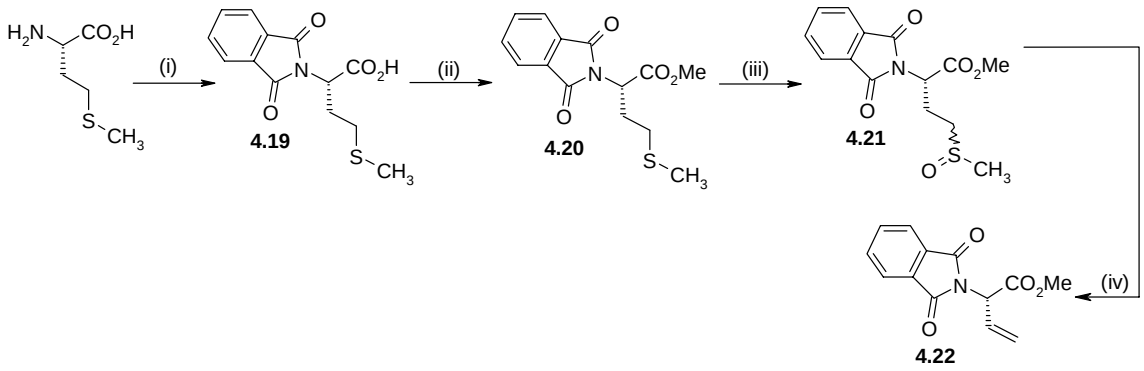
Molecular modelling indicates that the *E*-isomer is of lower energy and as such this is presumed to be the thermodynamic product. Using thermal heating a 2.5:1 ratio of geometric

isomers was obtained and the thermodynamic *E*-isomer predominated. This is in agreement with the literature[18] where Grubbs second generation catalyst is known to favour the thermodynamic product. However, the use of microwave irradiation exclusively gave the kinetic product (*Z*-isomer). To the best of our knowledge this is the first reported example of this microwave effect in the area of RCM. The reason for this is postulated to be because microwave heating increases the reaction such that the kinetic product is exclusively favoured over the thermodynamic product.

The chromatographic separation of geometric isomers **4.16** and **4.17** is the only example of such a separation in this thesis. In all other RCM macrocyclisations the geometric isomers were inseparable using flash chromatography. As such the ratio of the isomers was quantified by NMR but as they were inseparable, and were used as such, it is not possible to assign which is the predominant isomer.

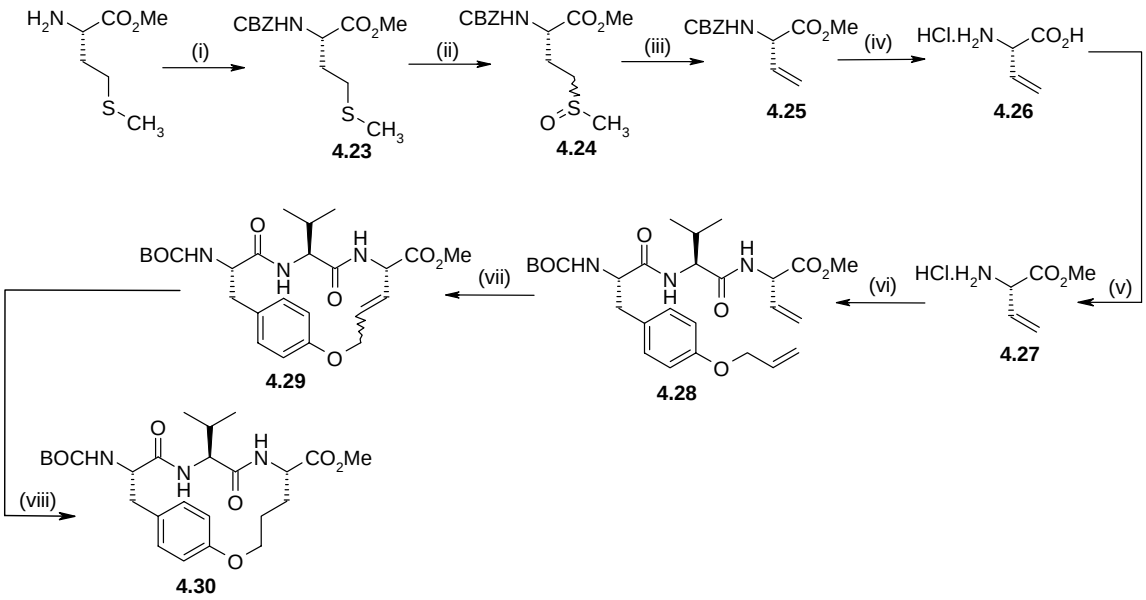
4.7: Synthesis of 16-membered ring Tyr-Val-Gly macrocycle

The synthesis of the sixteen membered ring Tyr-Val-Gly macrocycle required the preparation of (S)-vinyl-Gly-OMe (**4.27**). This was first attempted using literature precedence[19] starting from commercially available (*L*)-methionine (**Scheme 4.4**). The amino acid was condensed with phthalic anhydride using microwave irradiation to give carboxylic acid **4.19**. This was esterified with saturated methanolic hydrogen chloride to give *N*-phthaloyl-methionine methyl ester **4.20**. Oxidation with sodium metaperiodate afforded sulfoxide **4.21** as a 1:1 mixture of diastereoisomers. However, in my hands, the reported[20] photolysis failed to yield desired product **4.22**. In addition, thermal elimination did not yield the desired product. This reaction sequence was therefore abandoned.



Scheme 4.4. *Reagents and Conditions:* **(i)** Phthalic anhydride, H_2O, microwave, (100%); **(ii)** $HCl_{(g)}$, MeOH, (100%); **(iii)** $NaIO_4$, MeOH, H_2O, (94%); **(iv)** 1600W photolysis, MeCN, (0%); or Xylene, reflux, (0%)

The required (S)-vinyl-Gly-OMe **4.27** was successful synthesised as shown in **Scheme 4.5**. Commercially available Met-OMe was orthogonally protected to give *N*-CBZ amino acid **4.23**. Oxidation with sodium metaperiodate gave sulfoxide **4.24** as a 1:1 mixture of diastereoisomers. This was thermally eliminated under vacuum following a literature procedure,[21] and the distillate treated with 6M aqueous hydrochloric acid, at reflux, to give vinyl amino acid **4.26**. Acid catalysed esterification furnished the hydrogen chloride salt of amine **4.27**. Peptide coupling of this to previously prepared dipeptide carboxylic acid **4.13,** using HATU methodology gave diene **4.28**. This was ring closed using microwave irradiation to yield macrocycle **4.29** as a 1:3.8 mixture of geometric isomers by NMR. These isomers were not separable and were subjected to hydrogenation at atmospheric pressure and room temperature to give the di-protected saturated macrocycle **4.30**.



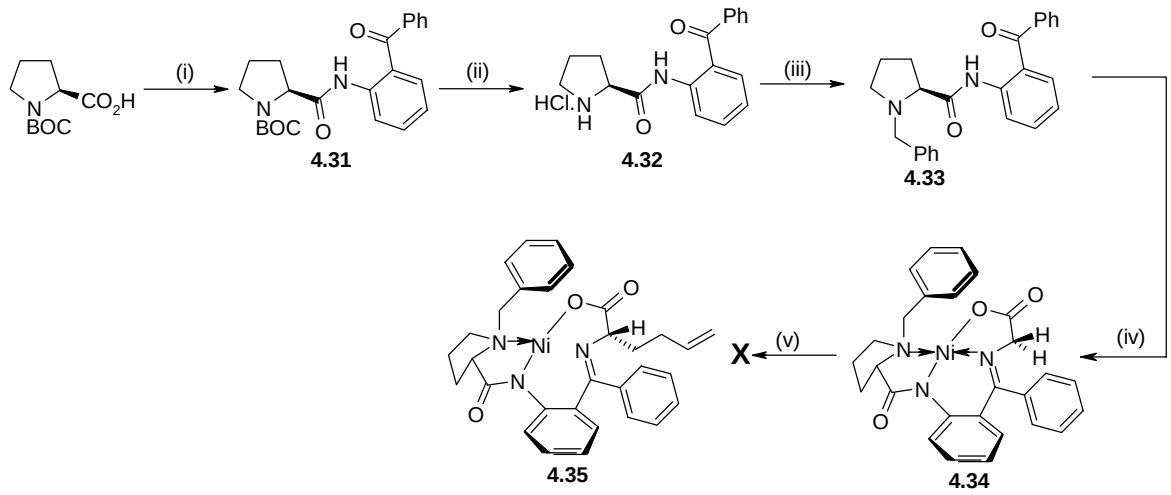
Scheme 4.5. *Reagents and Conditions:* **(i)** Benzyl chloroformate, $NaHCO_3$, EtOAc, H_2O, (98%); **(ii)** $NaIO_4$, H_2O, MeOH, (100%); **(iii)** thermal elimination (kugelrohr), (20%); **(iv)** 6M $HCl_{(aq)}$, (71%); **(v)** $SOCl_2$, MeOH, (100%); **(vi)** HATU, DIPEA, **4.13**, DMF, (76%); **(vii)** or 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (32%); **(viii)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (52%)

4.8: Attempted synthesis of 18-membered ring Tyr-Leu-Gly macrocycle of using homo-allyl glycine

The synthesis of the eighteen membered ring analogue of **4.18** and **4.30** using the established synthetic methodology described in Schemes **4.3** and **4.5** required the preparation of (S)-homo-allyl-Gly-OMe. As shown in **Scheme 4.6** this was attempted using transition metal coordination chemistry following literature procedures.[22]

Commercially available *N*-BOC-(L)-proline was coupled with 2-aminobenzophenone using HATU to give **4.31**. The BOC protecting group was cleaved using TFA in DCM and subsequent salt exchange achieved by neutralisation, organic extraction and treatment with ethereal hydrogen chloride gave the hydrochloride salt of amine **4.32**. This was *N*-benzylated using benzyl bromide to afford **4.33**. Preparation of the Gly-Ni(II)-BPB complex **4.34** was

achieved by reacting **4.33** with nickel (II) nitrate hexahydrate and glycine under basic conditions. However, allylation of this using potassium hydroxide as base under reaction conditions developed by Gu *et al*[23] afforded a complex mixture of products. NMR and MS analysis of the crude product indicated the desired compound was present but its purification and isolation was very problematic. This result motivated development of alternative methodology for the synthesis of macrocycle **4.42 (Scheme 4.7).** The reaction sequence in **Scheme 4.6** was not pursued further.

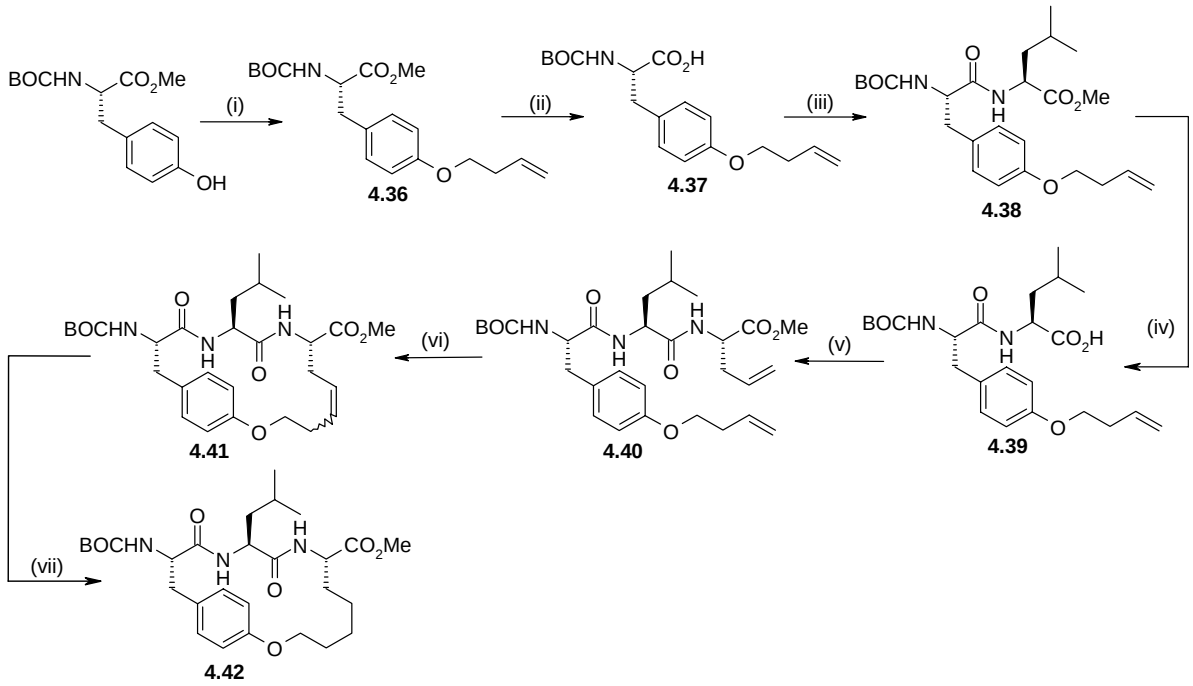


Scheme 4.6. *Reagents and Conditions:* **(i)** HATU, DIPEA, 2-aminobenzophenone, DMF, (69%); **(ii)** a) 10% TFA, DCM, b) HCl, Et_2O (100%); **(iii)** benzyl bromide, Et_3N, THF, (72%); **(iv)** KOH, $Ni(NO_3)_2.6H_2O$, glycine, MeOH, (42%); **(v)** NaOH, 4-bromo-but-1-ene, DMF, (0%)

4.9: Synthesis of 18-membered ring Tyr-Leu-Gly macrocycle

As shown in **Scheme 4.7** macrocycle **4.42** was prepared using slightly different methodology to that shown in **Schemes 4.3** and **4.5**. As the synthesis of (S)-homo-allyl-Gly-OMe was problematic instead of varying the chain length of the amine to vary the macrocyclic ring size, an alternative strategy of changing the length of the allyl chain on the tyrosine oxygen was used.

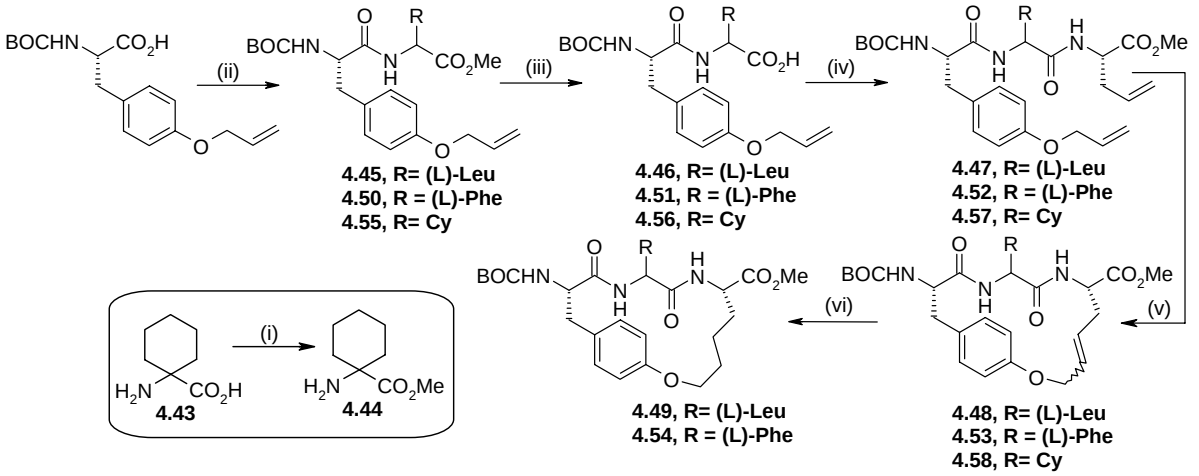
N-BOC-O-homo-allyl-Tyr-OMe **4.36** was prepared by allylation of commercial *N*-BOC-Tyr-OMe with 4-bromo-but-1-ene. This allowed the larger homologue of the 17-membered macrocycle **4.18** to be synthesised using (S)-allyl-Gly-OMe, thus negating the requirement to synthesise (S)-homo-allyl-Gly-OMe. Base hydrolysis of methyl ester **4.36,** followed by a HATU peptide coupling with Leu-OMe, gave dipeptide **4.38**. Sodium hydroxide hydrolysis gave carboxylic acid **4.39** which was coupled with (S)-allyl-Gly-OMe, using standard HATU reaction conditions, to give diene **4.40**. RCM was achieved using Grubbs second generation catalyst, microwave irradiation and a Lewis acid additive (chloro-dicyclohexylborne) to afford unsaturated macrocycle **4.41** in quantitative yield as a 1:1.9 mixture (by NMR) of geometric isomers. These were not separated and the mixture was hydrogenated under standard conditions to yield the saturated orthogonally protected macrocycle **4.42** in moderate yield.



Scheme 4.7. *Reagents and Conditions:* **(i)** K_2CO_3, 4-bromo-but-1-ene, DMF, (27%); **(ii)** NaOH, THF, H_2O, MeOH, (85%); **(iii)** HATU, DIPEA, Leu-OMe, DMF, (64%); **(iv)** NaOH, THF, H_2O, MeOH, (96%); **(v)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (84%); **(vi)** 3 x 10 mol% **3.9**, 10 mol% chlorodicyclohexylborane, 1,1,2-TCE, microwave, (100%); **(vii)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (26%)

4.10: Synthesis of 17-membered ring Tyr-Leu-Gly, Tyr-Phe-Gly, Tyr-cyclohexane-Gly macrocycles

As shown in **Scheme 4.8** a range of macrocycles **4.49, 4.54** and **4.58** were prepared with (L)-leucine, (L)-phenylalanine and cyclohexane in the P2 position to investigate the effect of changing the P2 amino acid. Leu-OMe and Phe-OMe were commercially available, however 1-amino-cyclohexane-1-methyl ester (**4.44)** was prepared from its corresponding amino acid (**4.43)** using acid catalysed esterification following a literature precedence.[24] Each of these methyl esters was coupled to commercially available *N*-BOC-Tyr-O-Allyl-H, using HATU peptide coupling methodology, to give dipeptides **4.45, 4.50** and **4.55**. Each of these was hydrolysed under basic conditions, and the resultant carboxylic acids **4.46**, **4.51** and **4.56** coupled with (S)-allyl-Gly-OMe to give their respective dienes (**4.47, 4.52** and **4.57**). These three dienes were ring closed using Grubbs second generation catalyst and microwave irradiation. To the RCM reactions of the leucine and cyclohexane dienes (**4.47** and **4.57**) 10 mol% of Lewis acid chloro-dicyclohexylborane was also added. This was not added to the phenylalanine RCM reaction as the Lewis acid methodology was not established at that time. These RCM reactions afforded the three unsaturated macrocycles **4.48, 4.53 and 4.58** as mixtures of geometric isomers. For R = Leu, 1:9 ratio, R = Phe, 1:6.25 ratio and R = Cy, 1:6.5 ratio. The leucine and phenylalanine macrocycles (**4.48** and **4.53**) were then hydrogenated under standard conditions to yield saturated macrocycles **4.49** and **4.54**. Cyclohexane macrocycle **4.58** was not hydrogenated as molecular modelling indicated this was a very poor β-strand mimetic and as such no further work was performed on this macrocycle.



Scheme 4.8. *Reagents and Conditions:* **For R = Leu (ii)** HATU, DIPEA, Leu-OMe, DMF, (80%); **(iii)** NaOH, THF, H_2O, MeOH, (97%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (97%); **(v)** 3 x 10 mol% **3.9**, 10 mol% chloro-dicyclohexyl borane, 1,1,2-TCE, microwave, (91%); **(vi)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (98%).

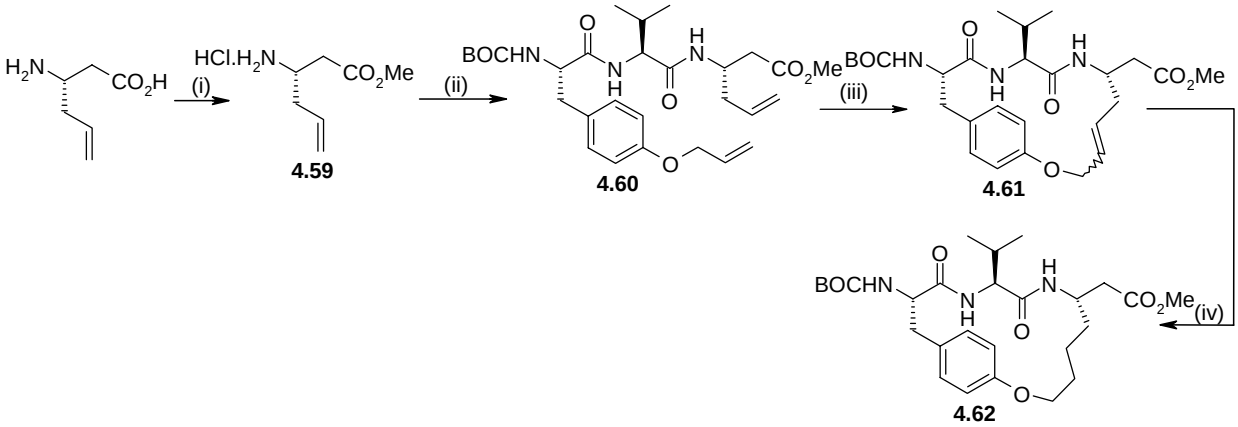
For R = Phe (ii) HATU, DIPEA, Phe-OMe, DMF, (81%); **(iii)** NaOH, THF, H_2O, MeOH, (93%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (65%); **(v)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (51%); **(vi)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (25%).

For R = Cy (i) $SOCl_2$, MeOH, (100%); **(ii)** HATU, DIPEA, **4.44** , DMF, (59%); **(iii)** NaOH, THF, H_2O, MeOH, (98%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (76%); **(v)** 3 x 10 mol% **3.9**, 10 mol% chloro-dicyclohexyl borane, 1,1,2-TCE, microwave, (31%).

4.11: Synthesis of 17-membered ring Tyr-Val-β-Gly macrocycle

Molecular modelling studies using the induced fit protocol (see **Section 5.1**) indicated that although the macrocycles created in the *in-silico* library (see **Section 4.2**) were excellent β-strand mimics in each case the distance between the electrophilic warhead and the active site cysteine was slightly longer than that of the acyclic β-strand calpain inhibitors described in **Chapter 2.** As such it was postulated that the use of a β-amino acid warhead would reduce this distance as the extra methylene group would extend the aldehyde slightly further away from the β-strand backbone. It was anticipated that this would in turn impart better calpain inhibition to the macrocycle. As shown in **Scheme 4.9** the synthesis of such a macrocycle (**4.61**) was achieved starting with commercially available (*S*)-3-amino-hex-5-enoic acid. This was esterified using methanolic hydrogen chloride to give the C-protected β-amino acid **4.59**.

HATU mediated peptide coupling with previously prepared carboxylic acid **4.13** gave diene **4.60** which was cyclised to give **4.61** as a mixture of geometric isomers (1:9.1) on treatment with Grubbs second generation catalyst and microwave irradiation. Hydrogenation under standard conditions gave the required saturated orthogonally protected β-amino acid macrocycle **4.62**.



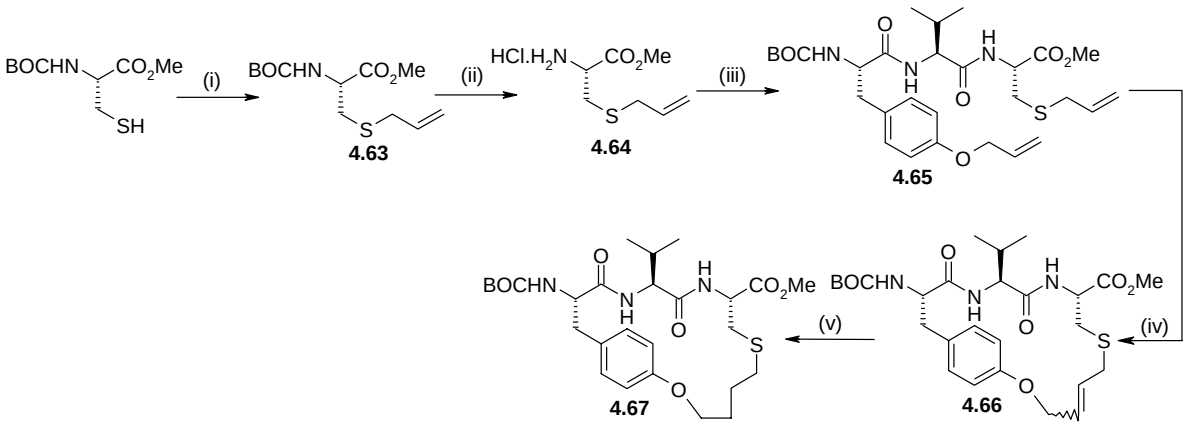
Scheme 4.9. *Reagents and Conditions:* **(i)** $SOCl_2$, MeOH, (86%); **(ii)** HATU, DIPEA, **4.13**, DMF, (83%); **(iii)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (66%); **(iv)** H_2, 20 mol% Pd/C, MeOH, (84%).

4.12: Synthesis of 19-membered ring Tyr-Val-Cys-macrocycle

As shown in **Schemes 4.10 – 4.13** a series of P1 analogues of **4.18 (**compounds **4.66**, **4.71**, **4.75**, and **4.80)** were selected for synthesis based on their excellent β-strand scores from *in-silico* conformational analysis (**Section 4.3**).

Scheme 4.10 details the synthesis of macrocycle **4.67** which incorporates cysteine at the P1 position. The synthesis of allyl cysteine was achieved following literature procedures.[25] Commercially available *N*-BOC-Cys-OMe was allylated with allyl bromide using triethylamine as a mild base to give orthogonally protected allyl cysteine **4.63** which was treated with a saturated ethereal hydrogen chloride solution to give the hydrogen chloride salt of amine **4.64**. Coupling with previously prepared carboxylic acid **4.13**, using HATU peptide coupling methodology, furnished diene **4.65**. Ring closure was achieved using Grubbs second generation catalyst and microwave irradiation to afford unsaturated macrocycle **4.66**

as a 1:1.7 mixture of geometric isomers. The saturated macrocycle **4.67** was obtained by hydrogenation of **4.66** at atmospheric pressure and room temperature.

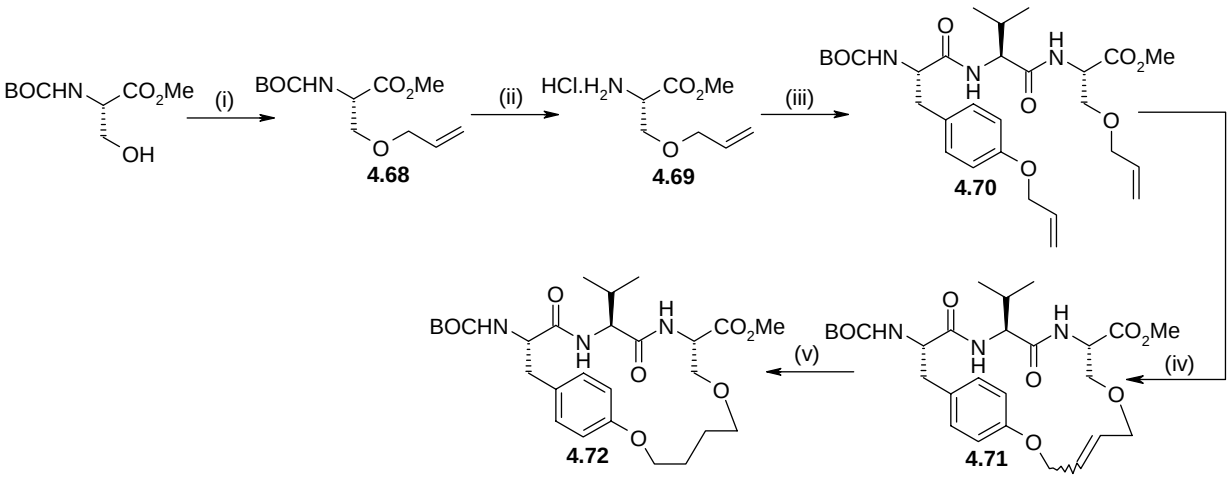


Scheme 4.10. *Reagents and Conditions:* **(i)** Et_3N, allyl bromide, DCM, (55%); **(ii)** $HCl_{(g)}$, Et_2O, (100%); **(iii)** HATU, DIPEA, **4.13**, DMF, (97%); **(iv)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (72%); **(v)** H_2, 20 mol% Pd/C, MeOH, EtOAc (44%).

4.13: Synthesis of 19-membered ring Tyr-Val-Ser-macrocycle

Scheme 4.11 describes the synthesis of macrocycle **4.72** which contains serine at the P1 position. Allylation of commercially available *N*-BOC-Ser-OMe proved to be more problematic than allylation of the direct cysteine analogue (**4.63**). Attempted base mediated allylation, using either sodium hydride or triethylamine as the base, with allyl bromide resulted in no reaction and only starting material was recovered in quantitative yield. However, allylated serine (**4.68)** was successfully obtained under neutral conditions using a literature procedure.[26] First the mixed anhydride of allyl alcohol and ethyl chloroformate was prepared (allyl ethyl carbonate) and this was reacted with *N*-BOC-Ser-OMe, triphenylphosphine and allyl palladium (II) chloride dimer, via a Π-allylpalladium complex intermediate, to give orthogonally protected allyl serine **4.68**. The *N*-BOC protecting group was cleaved using a 4M solution of hydrogen chloride in 1,4-dioxane to give the hydrogen chloride salt of amine **4.69**. This was coupled with previously synthesised carboxylic acid **4.13** using HATU mediated peptide coupling to give diene **4.70**. RCM using Grubbs second generation catalyst and microwave irradiation gave unsaturated macrocycle **4.71** as a 1:4.7

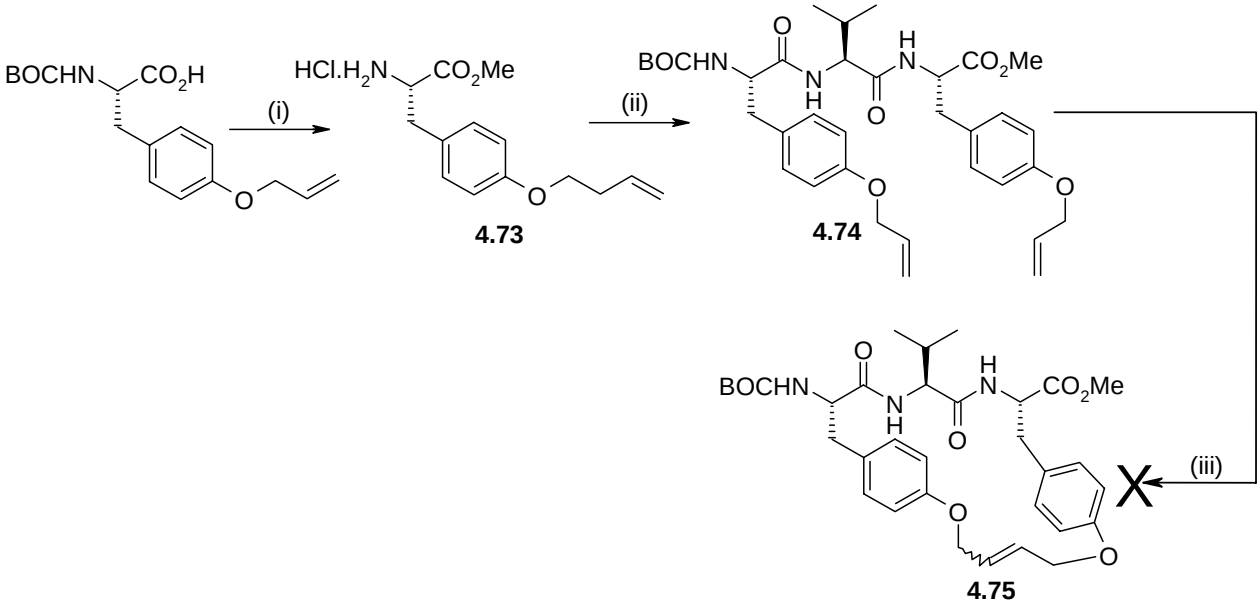
mixture of geometric isomers. Hydrogenation of this using palladium on carbon catalyst gave saturated macrocycle **4.72.**



Scheme 4.11. *Reagents and Conditions:* **(i)** NaH, allyl bromide, DMF (0%); or Et_3N, allyl bromide, DCM (0%); or a) allyl alcohol, ethyl chloroformate, Et_3N, Et_2O b) allyl palladium chloride, PPh_3, THF (21%); **(ii)** 4M HCl, Dioxan, (87%); **(iii)** HATU, DIPEA, **4.13**, DMF, (62%); **(iv)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (23%); **(v)** H_2, 20 mol% Pd/C, MeOH, EtOAc (100%).

4.14: Attempted synthesis of 23-membered ring Tyr-Val-Tyr-macrocycle

An attempted route to the synthesis of the P1 tyrosine analogue of **4.18** is shown in **Scheme 4.12.** The required O-allyl-(L)-Tyr-OMe (**4.73**) was prepared from commercially available *N*-BOC-O-allyl-(L)-Tyr-H using an acidic methanolic solution to achieve a simultaneous *N*-BOC cleavage and esterification reaction in one pot. Treatment of the resultant hydrochloride salt of amine **4.73** with carboxylic acid **4.13** and HATU gave diene **4.74**. RCM of this diene using using Grubbs second generation catalyst and microwave irradiation did not yield any ring closed product. Only starting material was recovered and as such the reaction sequence was abandoned.



Scheme 4.12. *Reagents and Conditions:* **(i)** $SOCl_2$, MeOH, (96%); **(ii)** HATU, DIPEA, **4.13**, DMF, (17%); **(iii)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (0%).

4.15: Attempted synthesis of 21-membered ring Tyr-Val-Gln-macrocycle

An attempted route to the synthesis of the P1 glutamine analogue of **4.18** is shown in **Scheme 4.13.** Commercially available Glu-OMe was *N*-BOC protected using BOC anhydride and triethylamine to afford orthogonally protected amino acid **4.76** using an established procedure.[27] The side chain acid of this was coupled with allyl amine and HATU to give the required diprotected allyl Gln **4.77**. The *N*-BOC protecting group was cleaved using saturated ethereal hydrogen chloride to give the hydrogen chloride salt of amine **4.78**. This was reacted with dipeptide carboxylic acid **4.13** and HATU to yield diene **4.79**. However, ring closure of this diene using Grubbs second generation catalyst and microwave irradiation did not yield the desired macrocycle. As discussed in **Sections 4.16** and **4.20** this is postulated to be due to the formation of a catalyst deactivating chelate. No further work was performed on this macrocyclic system.

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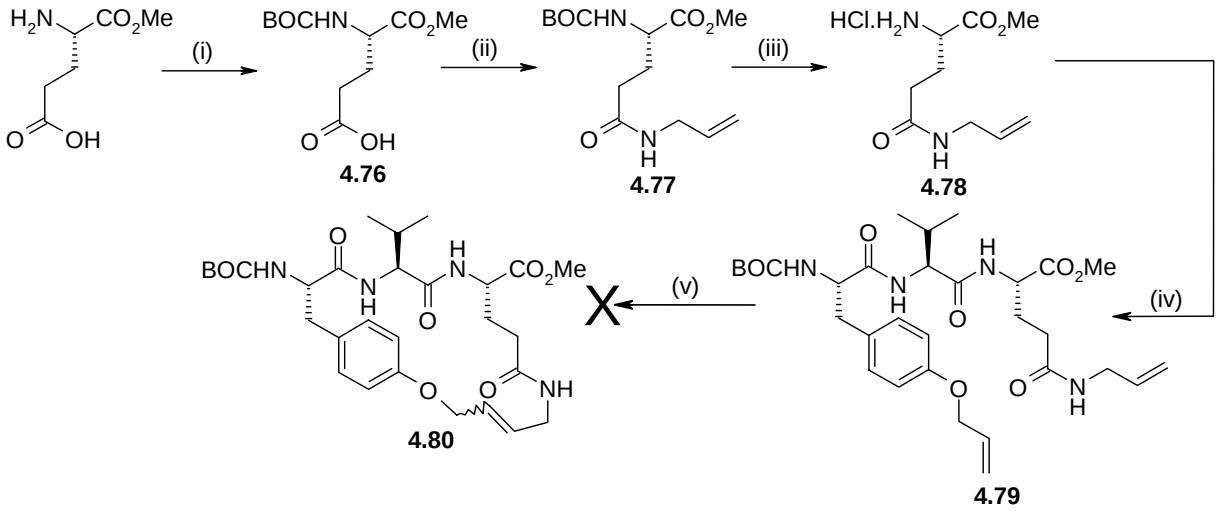


Scheme 4.13. *Reagents and Conditions:* **(i)** $(BOC)_2O$, Et_3N, Dioxan, H_2O, (97%); **(ii)** HATU, DIPEA, allyl amine, DMF, (14%); **(iii)** $HCl_{(g)}$, Et_2O, (100%); **(iv)** HATU, DIPEA, **4.13**, DMF, (38%); **(v)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (0%).

4.16: Effect of substrate chelation on RCM

A number of dienes both within this thesis and the Abell research group in general have failed to ring close when subjected to RCM. Analysis of these dienes reveals a common theme (**Figure 4.6**).

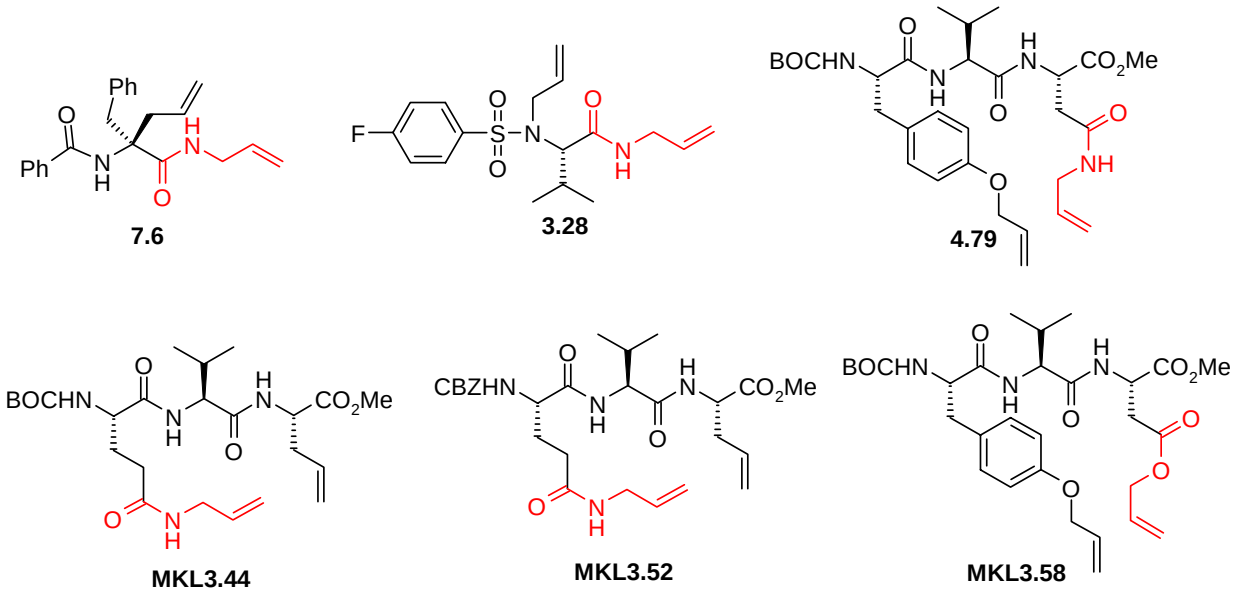


Figure 4.6: Dienes which fail to cyclise using RCM

Generally dienes that contain either an allyl amide or allyl ester result in either low yields of ring closed product or no ring closed product at all. This observation leads us to propose that

dienes of this type form a stable, catalyst deactivating, six membered chelate during the RCM catalytic cycle (**Figure 4.7**) for which there is some precedence.

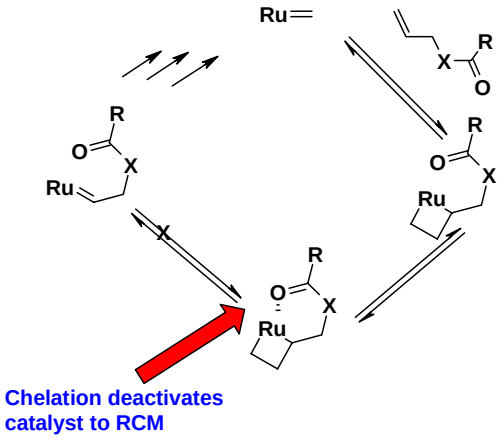


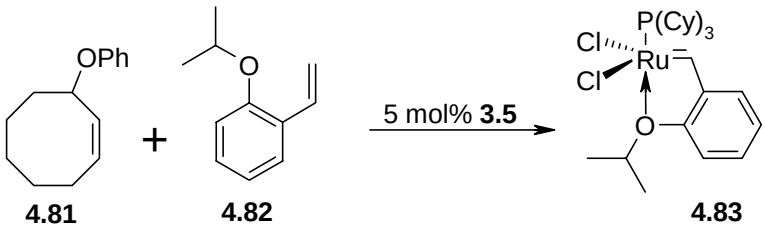
Figure 4.7: Proposed catalyst deactivating chelate

The chelation of oxygen functionalities to ruthenium during metathesis was recognised very early by both Grubbs *et al.*[28] and Schrock *et a.l*[29] during the development of RCM methodology. However, such chelates are not well documented in the literature. Often, as discussed in **Section 7.1**, the failure of RCM in substrates of this type is assigned to the fact that these dienes adopt a conformation not conducive to cyclisation, i.e. an extended conformation across the C1-N1 amide bond with respect to the rest of the molecule (**Figure 7.1**). It is proposed in this thesis that this is not the primary reason why such dienes fail to undergo RCM. Instead it is suggested that the reason why such substrates fail to ring close is the result of chelate formation.

This thesis also proposes that there is a growing body of evidence related to the existence of such chelates, and that these are indeed catalyst deactivating chelates. There are three particularly pertinent examples of published work which support this hypothesis;

1. As shown in **Scheme 4.14** Hoveyda *et al*[30, 31] serendipitously isolated a stable, catalytically active species (**4.83)** from the attempted ring opening metathesis, cross metathesis reaction of 2-isopropoxystyrene (**4.82**) with octadiene (**4.81)** in the

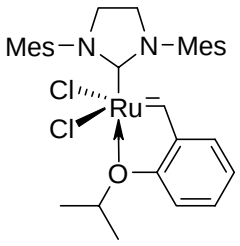
presence of Grubbs first generation catalyst (**3.5)**. Complex **4.83** was active in metathesis reactions and was of sufficient stability to be recovered efficiently by chromatography. This clearly demonstrates the potential of intramolecular chelating effects to interfere dramatically with a metathesis reaction.



4.81 **4.82** **4.83**

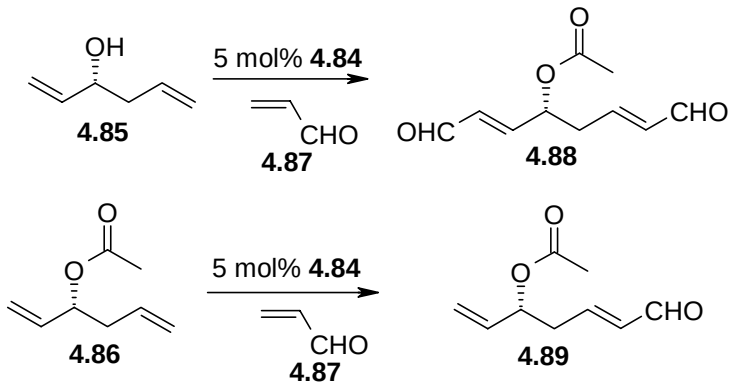
Scheme 4.14: Isolation of **4.83**

2. This chelation principle was extended to prepare, by ligand exchange, the chromatographically stable Grubbs second generation catalyst analogue **4.84.**[32, 33] This work proves that Grubbs second generation catalyst (**3.9**) is also capable of participating in a chelation reaction.



4.84

3. Chelate formation has been used in cross metathesis to successfully to obtain chemoselectivity (**Scheme 4.15**).[34, 35] The cross metathesis of an hydroxy-olefin **4.85** and an acetoxy-olefin **4.86** with acrolein **4.87** under identical conditions yielded different products. Alcohol **4.85** was functionalised twice to afford **4.88** whereas **4.86** yielded only the product of a single cross coupling **4.89**.



Scheme 4.15: Chemo-selective cross metathesis

It was proposed[35] that selectivity could arise through either deactivation of one of the carbon-carbon double bonds by the electron-withdrawing acetoxy group or, as work within our laboratory in the field of RCM would suggest, through the formation of an unreactive six-membered catalyst deactivating chelate (**Figure 4.8**). This complex results in selective cross metathesis of the homo-allylic unit in **4.86** only. This is because any chelate derived from this would require seven-membered ring formation. As such this would be expected to be much less stable than the six membered ring chelate from the allylic moiety. In the case of hydroxy substrate **4.85** no such stable ring chelates are possible, and thus unselective CM is observed.

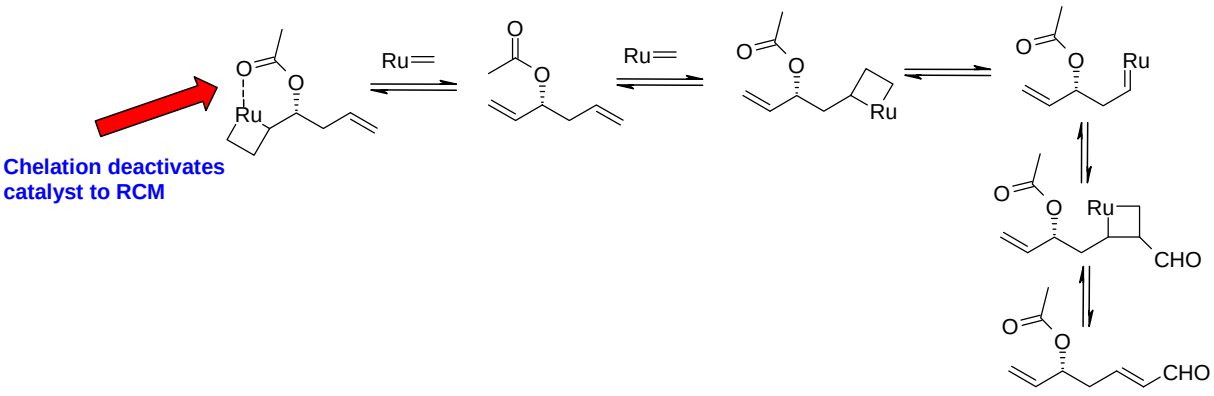
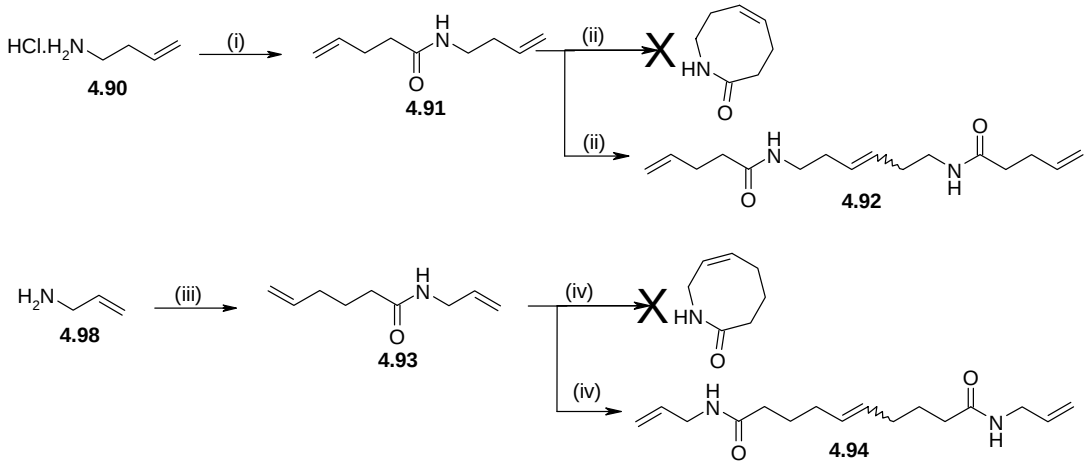


Figure 4.8: CM chemoselectivity from chelate formation.

4.17: Design and synthesis of a model system to understand chelation effects in RCM

As described in **Section 4.16** allyl amides or allyl esters are problematic RCM substrates as they form catalyst deactivating chelates. As such a test system to establish if the heteroatom

(i.e the nitrogen or oxygen in the amide or ester) is the key constituent of a substrate that forms a catalyst deactivating chelate was devised. As shown in **Scheme 4.16** the two amide dienes **4.91** and **4.93** were prepared by HATU mediated peptide coupling of 5-hexenoic acid/allyl amine and 4-pentenoic acid/homo-allyl amine, respectively.



Scheme 4.16. *Reagents and Conditions*: **(i)** HATU, DIPEA, 4-pentenoic acid, DMF, (78%); **(ii)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (0%); **(iii)** HATU, DIPEA, 5-hexenoic acid, DMF, (75%); **(iv)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (0%).

The RCM substrates (**4.91** and **4.93**) are both capable of forming six membered chelates. However, the six membered chelate formed by **4.93** results from the allyl amide moiety and as such is a chelate containing an heteroatom whereas the six membered chelate formed by **4.91** results from the allyl moiety and as such the chelate contains no heteroatom (**Figure 4.9)**

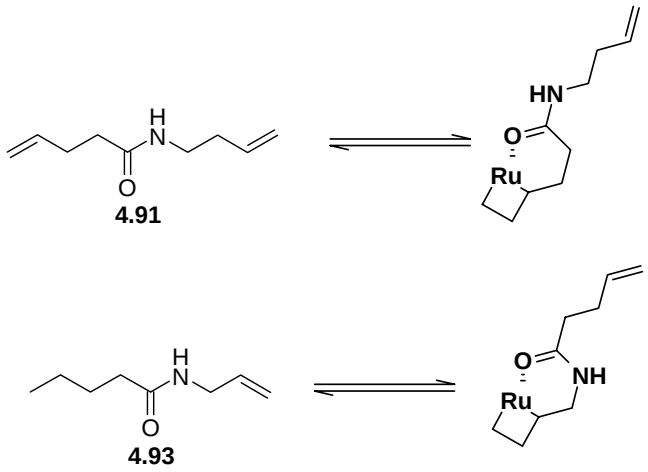


Figure 4.9: Chelates formed by amides **4.91** and **4.93**

If the presence of the heteroatom was of primary importance to forming the stable catalyst deactivating chelate then **4.91** would be expected to ring close whereas **4.93** would not. However, as shown in **Scheme 4.16** neither of these substrates yielded ring closed product and instead an intermolecular CM reaction occurred. This reaction was characterised by LC-MS analysis and presumably the cross metathesis reaction involved the olefins that do not participate in six membered ring chelation. This test system therefore suggests that the ability to form a six membered chelate is the dominant effect rather than the presence of a heteroatom in the chain that forms the chelate. This knowledge was then used to improve the yields of the macrocycles as discussed in **Section 4.19**.

4.18: Strategies to avoid catalyst deactivation from substrate chelation

With this chelation hypothesis in hand it was deemed necessary to either design new synthetic strategies or develop methodology, which would avoid or destabilise chelate formation. The two strategies devised were the use of a Lewis acid to make the chelate more labile (**Section 4.19**) and the redesign of a synthetic route to ensure there are no dienes which are capable of chelation formation (**Section 4.20**).

4.19: Use of a Lewis acid as an additive in RCM reactions

If chelate formation is a problem a classical method to circumvent such an issue is to add a Lewis acid to destabilise the chelate. As such it was postulated that if a Lewis acid was added to the RCM reaction media then this should destabilise the catalyst deactivating chelate and thus facilitate productive RCM. However, one major concern was that the additive must also be compatible with the RCM catalyst and that it must also undergo kinetically labile coordination with the carbonyl group. During early metathesis methodology development Furstner *et al*[36] demonstrated that strong Lewis acids such as $TiCl_4$ or $SnCl_4$ decomposed metathesis catalysts, whereas $Ti(O^iPr)_4$ was used successfully to increase RCM reaction

yields. Furthermore, Vedrenne *et al*[37] studied a number of Lewis acids to improve the yields of CM reactions, where one of the partners was an allylic carbamate or amide. From this study, it was determined that boron-based Lewis acids were better candidates for promoting metathesis reactions than titanium-based Lewis acids. In addition, chloro-dicyclohexyl borane was the best of the boron-based Lewis acids studied and as such this was adopted as the Lewis acid additive in our work.

This strategy was investigated using five different dienes. As shown in **Table 4.3** in all five reactions where chloro-dicyclohexyl borane was added an increase in yield was observed.

Furthermore, the stoichiometry of Lewis acid required to obtain the maximum increase in yield (compound **6.4**) agreed with those previously published[37] in the CM area. Optimum yields were obtained using a sub equimolar quantity of the Lewis acid.

Moreover, the concept that addition of a Lewis acid to a diene which fails to ring close under microwave irradiation conditions would then facilitate this to do so was validated by another member of the group. Compound **MKL 2.4** was isolated, albeit in low yield, when Lewis acid was added. Without addition of Lewis acid no ring closed product was obtained.

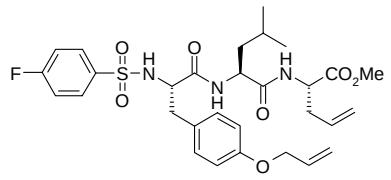
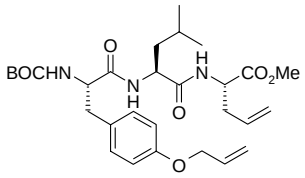
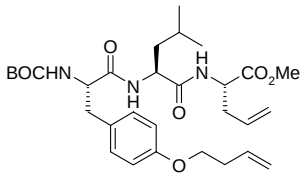
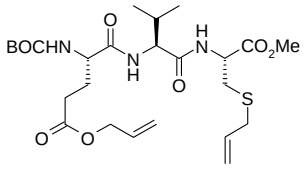
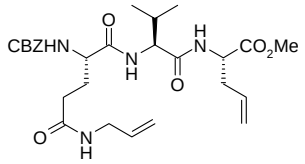
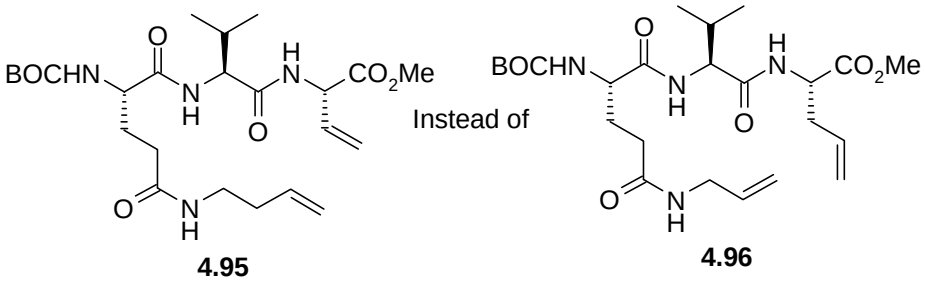
Compound	Compound number	Reaction conditions	Yield (%)
	**6.4**	3 X 10 mol% **3.9**, µ-wave	29
		3 X 10 mol% **3.9**, 10 mol% $(Cy)_2BCl$, µ-wave	65
		3 X 10 mol% **3.9**, 100 mol% $(Cy)_2BCl$, µ-wave	38
	**4.47**	3 X 10 mol% **3.9**, µ-wave	43
		3 X 10 mol% **3.9**, 10 mol% $(Cy)_2BCl$, µ-wave	91
	**4.40**	3 X 10 mol% **3.9**, µ-wave	N/A
		3 X 10 mol% **3.9**, 10 mol% $(Cy)_2BCl$, µ-wave	100
	**MKL 2.7**	3 X 10 mol% **3.9**, µ-wave	42
		3 X 10 mol% **3.9**, 10 mol% $(Cy)_2BCl$, µ-wave	50
	**MKL 2.4**	3 X 10 mol% **3.9**, µ-wave	0
		3 X 10 mol% **3.9**, 10 mol% $(Cy)_2BCl$, µ-wave	14

Table 4.3: Effect of Lewis acid additive on the yield of RCM

4.20: Chain length alteration to avoid catalyst deactivation from substrate chelation

As described in **Section 4.18** a strategy to circumnavigate the formation of a catalyst deactivating chelate is to ensure that the synthetic route avoids the formation of a stable chelate. An example of the modification of a synthetic route to avoid possible chelate formation is shown in **Figure 4.10**. As previously described, diene **4.96** did not undergo RCM. This was postulated to be because diene **4.96** forms a stable catalyst deactivating

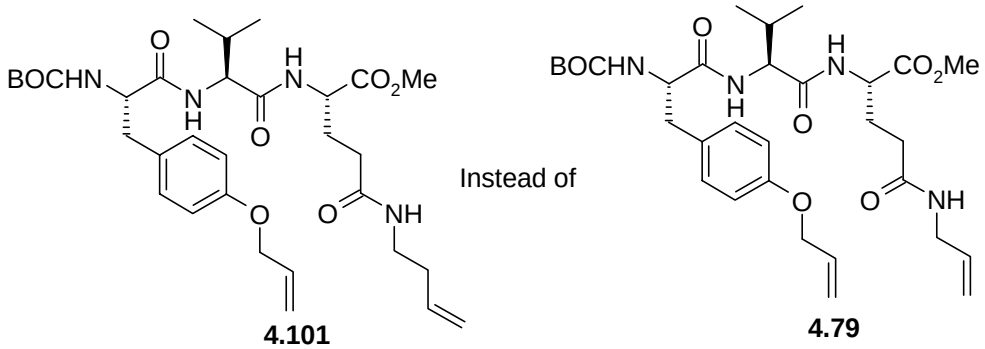
chelate. A strategy envisaged to circumnavigate this problem was to change the chain length of each olefin (i.e **4.95** compared to **4.96**) so that chelate formation is no longer possible. RCM of **4.95** would yield the same macrocyclic product (after subsequent hydrogenation). However, synthesis of this exact example was not performed as it required significant synthetic effort to prepare the required precursors to **4.95**.



4.95 **4.96**

Figure 4.10: Avoidance of chelation by changing chain length

In order to validate the concept illustrated in **Figure 4.10** the higher homologue of **4.80** was prepared (**Scheme 4.17**). As described in **Scheme 4.13,** the precursor diene to **4.80 (4.79)** failed to ring close because it forms a stable chelate. As shown in **Figure 4.11** chelate formation can be avoided by incorporation of an extra methylene group. The only difference between diene **4.79** and diene **4.101** is that **4.101** contains an extra methylene group so that **4.101** can no longer form a six membered chelate.

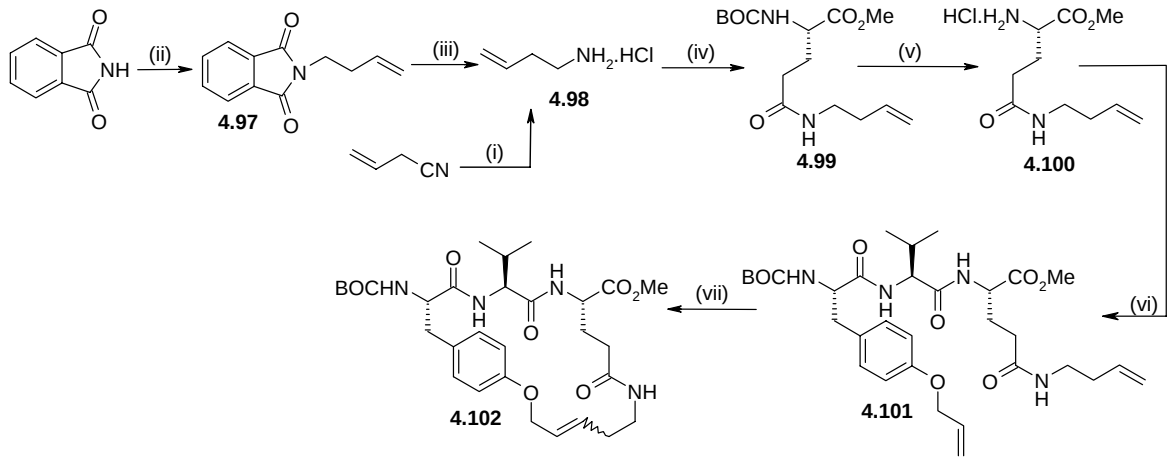


4.101 **4.79**

Figure 4.11: Avoidance of chelation by changing chain length

Before diene **4.101** could be synthesised the preparation of but-3-enylamine (**4.98**) was first required. This was successfully achieved using two different methods. In method one, allyl cyanide was reduced with a combination of lithium aluminium hydride and aluminium

trichloride to give amine **4.98** as the hydrochloride salt after work-up and subsequent treatment with ethereal hydrogen chloride. Although successful this was deemed to be potentially problematic on a large scale due to the toxicity of allyl cyanide. In addition, the aqueous work-up/isolation of **4.98** was not trivial. As such a second method was used, based on literature procedures,[38] to prepare large quantities of **4.98**. Potassium phthalimide, prepared from phthalimide using potassium hydroxide, was allylated with 4-bromo-1-butene to afford isoindole **4.97** which gave **4.98** on treatment of this with hydrazine. However, attempted coupling of **4.98** with commercially available *N*-BOC-Glu-OMe and HATU gave returned starting material only after aqueous work-up. However, EDC peptide coupling gave required amide **4.99**, albeit it in relatively low yield. Amine **4.100** was obtained by using acidic methanol to cleave the *N*-BOC protecting group of **4.99**. This was coupled to carboxylic acid **4.13** using standard EDC peptide coupling conditions to afford diene **4.101**. This was ring closed using Grubbs second generation catalyst and microwave irradiation to yield macrocycle **4.102** as a 1:2 mixture (by NMR) of geometric isomers. Modelling of this macrocycle indicated that this would not be a good β-strand mimic and as such no further synthesis was performed on this macrocycle. However, this reaction sequence validated the concept that changing the chain length of the olefin avoids chelate formation and allows productive RCM to be achieved.



Scheme 4.17. *Reagents and Conditions:* **(i)** $LiAlH_4$, $AlCl_3$, Et_2O, (54%); **(ii)** a) KOH, EtOH; b) 4-bromo-1-butene, DMF (96% over 2 steps); **(iii)** Hydrazine monohydrate, EtOH, (37%); **(iv)** HATU, DIPEA, *N*-BOC-Glu-OMe, DMF (0%); or EDC, HOBt.H_2O, DIPEA, *N*-BOC-Glu-OMe, DMF, (18%); **(v)** $SOCl_2$, MeOH, (100%); **(vi)** EDC, HOBt.H_2O, DIPEA, **4.13**, DMF, (54%); **(vii)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (17%).

4.21: Conclusions and further work

Novel calpain inhibitor motif **4.1** was devised. This motif was designed to conformationally constrain a tripeptide into a β-strand conformation whilst allowing it to form three critical β-strand hydrogen bonds to the enzyme. Retrosynthetic analysis of this template was undertaken and an *in-silico* combinatorial library of two hundred and eighty eight possible β-strand templates was prepared. Conformational analysis of this library was performed and from this a number of excellent β-strand templates were identified.

The synthesis of ten β-strand templates, chosen on the basis of their excellent percentage β-strand scores, was successfully achieved. However, the synthesis of two templates (**4.75** and **4.80**) using ring closing metathesis failed. The reason for this is postulated to be due to the formation of a six membered catalyst deactivating chelate.

New microwave irradiation methodology was developed for the ring closure of tripeptide dienes using RCM chemistry. Moreover the use of microwave irradiation resulted in an

interesting observation. In the only example where the chromatographic separation of geometric isomers (**4.16** and **4.17**) from the RCM reaction was possible, microwave irradiation resulted in the exclusive formation of the kinetic isomer. This is of particular note as Grubbs second generation is known to favour the thermodynamic product under non-microwave irradiation conditions. To the best of our knowledge this is the first reported example of such an effect. This certainly warrants further studies.

Two methods were identified to overcome the formation of a postulated six-membered catalyst deactivating chelate. Addition of Lewis acid chloro-dicyclohexyl borane to the RCM reaction media to destabilise the chelate and changing the chain length of the olefins to avoid chelate formation were both successful. In particular the use of a Lewis acid was extremely effective. In all cases where comparable data was obtained this resulted in an increase yield of ring closed product.

A vast amount of further work could be undertaken in this area. There are still a large number of novel β-strand templates, which were identified by conformational analysis and which could be synthesised using the synthetic methodologies developed in this Chapter.

Furthermore, factors that govern the geometric isomer ratio from the RCM reaction remain unknown. Studies to determine exactly what influences this ratio would be of interest to the wider synthetic chemistry community.

References for Chapter 4

1. Abell, A. D.; Brown, K. M.; Coxon, J. M.; Jones, M. A.; Miyamoto, S.; Neffe, A. T.; Nikkel, J. M.; Stuart, B. G., Synthesis and evaluation of eight-membered cyclic pseudo-dipeptides. *Peptides* **2005,** 26, (2), 251-258.

2. Ersmark, K.; Nervall, M.; Gutierrez-de-Teran, H.; Hamelink, E.; Janka, L. K.; Clemente, J. C.; Dunn, B. M.; Gogoll, A.; Samuelsson, B.; Aqvist, J.; Hallberg, A., Macrocyclic inhibitors of the malarial aspartic proteases plasmepsin I, II, and IV. *Bioorganic & Medicinal Chemistry* **2006,** 14, (7), 2197-2208.

3. Tyndall, J. D. A.; Reid, R. C.; Tyssen, D. P.; Jardine, D. K.; Todd, B.; Passmore, M.; March, D. R.; Pattenden, L. K.; Bergman, D. A.; Alewood, D.; Hu, S. H.; Alewood, P. F.; Birch, C. J.; Martin, J. L.; Fairlie, D. P., Synthesis, stability, antiviral activity, and protease-bound structures of substrate-mimicking constrained macrocyclic inhibitors of HIV-1 protease. *Journal of Medicinal Chemistry* **2000,** 43, (19), 3495-3504.

4. Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P., Beta-Strand Mimetics. *Chemical Reviews* **2004,** 104, (12), 6085-6117.

5. Kaul, R.; Surprenant, S.; Lubell, W. D., Systematic Study of the Synthesis of Macrocyclic Dipeptide beta-Turn Mimics Possessing 8-, 9-, and 10- Membered Rings by Ring-Closing Metathesis. *Journal of Organic Chemistry* **2005,** 70, (10), 3838-3844.

6. Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J., Microwave assisted organic synthesis - a review. *Tetrahedron* **2001,** 57, (45), 9225-9283.

7. Alterman, M.; Hallberg, A., Fast microwave-assisted preparation of aryl and vinyl nitriles and the corresponding tetrazoles from organo-halides. *Journal of Organic Chemistry* **2000,** 65, (23), 7984-7989.

8. de la Hoz, A.; Diaz-Ortis, A.; Moreno, A.; Langa, F., Cycloadditions under microwave irradiation conditions: Methods and applications. *European Journal of Organic Chemistry* **2000**, (22), 3659-3673.

9. Nilsson, P.; Gold, H.; Larhed, M.; Hallberg, A., Microwave-assisted enantioselective Heck reactions: Expediting high reaction speed and preparative convenience. *Synthesis-Stuttgart* **2002**, (11), 1611-1614.

10. Bremberg, U.; Lutsenko, S.; Kaiser, N. F.; Larhed, M.; Hallberg, A.; Mobert, C., Rapid and stereoselective C-C, C-O, C-N and C-S couplings via microwave accelerated palladium-catalyzed allylic substitutions. *Synthesis-Stuttgart* **2000**, (7), 1004-1008.

11. Larhed, M.; Moberg, C.; Hallberg, A., Microwave-accelerated homogeneous catalysis in organic chemistry. *Accounts of Chemical Research* **2002,** 35, (9), 717-727.

12. Kaiser, N. F. K.; Bremberg, U.; Larhed, M.; Moberg, C.; Hallberg, A., Fast, convenient, and efficient molybdenum-catalyzed asymmetric allylic alkylation under noninert conditions: An example of microwave-promoted fast chemistry. *Angewandte Chemie-International Edition* **2000,** 39, (20), 3596-3598.

13. Belda, O.; Moberg, C., Substituted pyridylamide ligands in microwave-accelerated Mo(0)-catalysed allylic alkylations. *Synthesis-Stuttgart* **2002**, (11), 1601-1606.

14. Mayo, K. G.; Nearhoof, E. H.; Kiddle, J. J., Microwave-accelerated ruthenium-catalyzed olefin metathesis. *Organic Letters* **2002,** 4, (9), 1567-1570.

15. Efskind, J.; Undheim, K., High temperature microwave-accelerated ruthenium-catalysed domino RCM reactions. *Tetrahedron Letters* **2003,** 44, (14), 2837-2839.

16. Grigg, R.; Martin, W.; Morris, J.; Sridharan, V., Synthesis of Delta(3)-pyrrolines and Delta(3)-tetrahydropyridines via microwave-accelerated ring-closing metathesis. *Tetrahedron Letters* **2003,** 44, (26), 4899-4901.

17. Aitken, S. G.; Abell, A. D., Olefin Metathesis: Catalyst Development, Microwave Catalysis, and Domino Applications. *Australian Journal of Chemistry* **2005,** 58, (1), 3-13.

18. Grubbs, R. H., *Handbook of metathesis*. Wiley-VCH Chichester, 2003.

19. Griesbeck, A. G.; Hirt, J., A photochemical route to vinylglycine and a vinylglycine dipeptide. *Liebigs Annalen* **1995**, (11), 1957-61.

20. Griesbeck, A. G.; Hirt, J., A Photochemical Route to Vinylglycine and a Vinylglycine Dipeptide. *Liebigs Annalen* **1995**, (11), 1957-1961.

21. Nudelman, A.; Marcovici-Mizrahi, D.; Nudelman, A.; Flint, D.; Wittenbach, V., Inhibitors of biotin biosynthesis as potential herbicides. *Tetrahedron* **2004,** 60, (8), 1731-1748.

22. Belokon, Y. N.; Tararov, V. I.; Maleev, V. I.; Savel'eva, T. F.; Ryzhov, M. G., Improved procedures for the synthesis of (S)-2-[N-(N'-benzyl-prolyl)amino]benzophenone (BPB) and Ni(II) complexes of Schiff's bases derived from BPB and amino acids. *Tetrahedron: Asymmetry* **1998,** 9, (23), 4249-4252.

23. Gu, X. Y.; Ndungu, J. A.; Qiu, W.; Ying, J. F.; Carducci, M. D.; Wooden, H.; Hruby, V. J., Large scale enantiomeric synthesis, purification, and characterization of omega-unsaturated amino acids via a Gly-Ni(II)-BPB-complex. *Tetrahedron* **2004,** 60, (37), 8233-8243.

24. Carrico, D.; Blaskovich, M. A.; Bucher, C. J.; Sebti, S. M.; Hamilton, A. D., Design, synthesis, and evaluation of potent and selective benzoyleneurea-based inhibitors of protein geranylgeranyltransferase-I. *Bioorganic & Medicinal Chemistry* **2005,** 13, (3), 677-688.

25. Goudreau, N.; Brochu, C.; Cameron, D. R.; Duceppe, J.-S.; Faucher, A.-M.; Ferland, J.-M.; Grand-Maitre, C.; Poirier, M.; Simoneau, B.; Tsantrizos, Y. S., Potent Inhibitors of the

Hepatitis C Virus NS3 Protease: Design and Synthesis of Macrocyclic Substrate-Based beta-Strand Mimics. *Journal of Organic Chemistry* **2004,** 69, (19), 6185-6201.

26. Kazmaier, U.; Maier, S.; Zumpe, F. L., Journey from amino acid and peptide chemistry to palladium and back. *Synlett* **2000**, (11), 1523-1535.

27. Manesis, N. J.; Goodman, M., Synthesis of a novel class of peptides: dilactam-bridged tetrapeptides. *Journal of Organic Chemistry* **1987,** 52, (24), 5331-41.

28. Fu, G. C.; Grubbs, R. H., Synthesis of Nitrogen-Heterocycles Via Catalytic Ring-Closing Metathesis of Dienes. *Journal of the American Chemical Society* **1992,** 114, (18), 7324-7325.

29. Feldman, J.; Murdzek, J. S.; Davis, W. M.; Schrock, R. R., Reaction of Neopentylidene Complexes of the Type M(Ch-T-Bu)(N-2,6-C6h3-I-Pr2)(or)2 (M = W, Mo) with Methyl Acrylate and N,N-Dimethylacrylamide to Give Metallacyclobutane Complexes. *Organometallics* **1989,** 8, (9), 2260-2265.

30. Kingsbury, J. S.; Harrity, J. P. A.; Bonitatebus, P. J.; Hoveyda, A. H., A recyclable Ru-based metathesis catalyst. *Journal of the American Chemical Society* **1999,** 121, (4), 791-799.

31. Harrity, J. P. A.; La, D. S.; Cefalo, D. R.; Visser, M. S.; Hoveyda, A. H., Chromenes through metal-catalyzed reactions of styrenyl ethers. Mechanism and utility in synthesis. *Journal of the American Chemical Society* **1998,** 120, (10), 2343-2351.

32. Gessler, S.; Randl, S.; Blechert, S., Synthesis and metathesis reactions of a phosphine-free dihydroimidazole carbene ruthenium complex. *Tetrahedron Letters* **2000,** 41, (51), 9973-9976.

33. Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H., Efficient and recyclable monomeric and dendritic Ru-based metathesis catalysts. *Journal of the American Chemical Society* **2000,** 122, (34), 8168-8179.

34. Connon, S. J.; Blechert, S., Recent developments in olefin cross-metathesis. *Angewandte Chemie-International Edition* **2003,** 42, (17), 1900-1923.

35. BouzBouz, S.; Cossy, J., Chemoselective cross-metathesis reaction. Application to the synthesis of the C1-C14 fragment of amphidinol 3. *Organic Letters* **2001,** 3, (10), 1451-1454.

36. Furstner, A.; Langemann, K., Total syntheses of (+)-ricinelaidic acid lactone and of (-)-gloeosporone based on transition-metal-catalyzed C-C bond formations. *Journal of the American Chemical Society* **1997,** 119, (39), 9130-9136.

37. Vedrenne, E.; Dupont, H.; Oualef, S.; Elkaim, L.; Grimaud, L., Dramatic effect of boron-based Lewis acids in cross-metathesis reactions. *Synlett* **2005**, (4), 670-672.

38. McNaughton, B. R.; Bucholtz, K. M.; Camaano-Moure, A.; Miller, B. L., Self-Selection in Olefin Cross-Metathesis: The Effect of Remote Functionality. *Organic Letters* **2005,** 7, (4), 733-736.

5.1: Use macrocyclic calpain inhibitors as anti-cataract agents

Chapter 4 described the synthesis of a range of macrocyclic β-strand mimics. With these in hand, the focus of the research was changed from the synthesis of β-strand mimics to the elaboration of these into a range of macrocyclic calpain inhibitors, which could potentially be used as anti-cataract agents. This chapter describes the synthesis of a range of macrocyclic calpain inhibitors with the objective of identifying a potential human anti-cataract therapeutic.

As described in **Section 1.4** calpain over-activation is thought to be involved in a number of human diseases of which cataract formation, and progression, is one example. There are a number of mechanisms by which cataracts are thought to be formed and subsequently develop. Calpain II mediated proteolysis is one which is currently receiving considerable attention in the literature.[1-4] As such the scientific rationale upon which our group embarked on designing and synthesising an anti-cataract agent is depicted schematically in **Figure 5.4**.

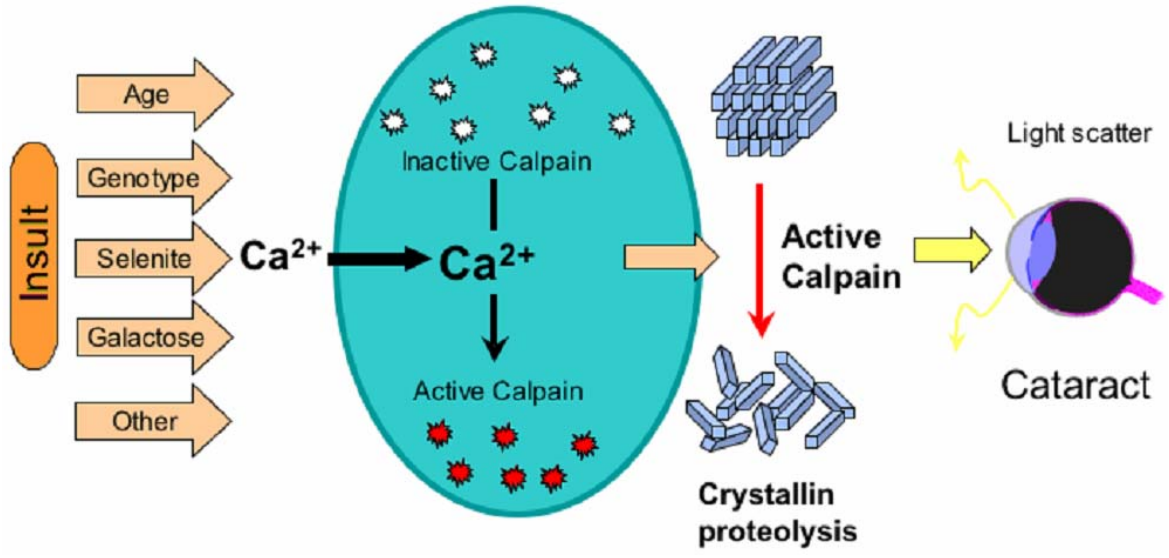


Figure 5.4: Schematic diagram to illustrate the role of calpain in cataract development

The hypothesis is that an "insult" to the eye, which can originate from many possible sources such as old age, diabetes, U.V. radiation and genetic factors amongst many others, results in increased levels of calcium in the eye. This increased calcium concentration activates lens

calpain (under homeostatic control this lens calpain is present in its inactive form). This calpain "over-activation", in turn leads to the proteolysis of crystallins.

There are four major families of crystallins, referred to as the alpha, beta, gamma and delta crystallins. In the lens of the eye, crystallins are water-soluble structural proteins and, under normal conditions, these crystallins are transparent and show a high degree of regular packing. However, proteolysis results in changes to the crystallin structure, which in turn leads to insolubilisation and aggregation. It is this insolubilisation that results in light scattering and the condition known as cataract. It is postulated that administration of a calpain inhibitor would reverse this over-activation of calpain, and hence stop the proteolysis of the crystallins, thus preventing or slowing down cataract formation/development.

There is significant evidence that such an approach could result in an effective anti-cataract drug. Over the past two decades, several published studies have suggested that calpain II is involved in animal cataract. Early investigations established that calpain II proteolysed crystallins and that calpain inhibitors could prevent the progression of rodent cataract induced by Ca^{2+} mediated activation of calpain II.[1, 5, 6] Taken together with more recent studies,[7, 8] it is now generally accepted that calpain II plays a major role in rodent lens opacification. Furthermore, several studies have shown calpain II to be the major calpain activated in murine diabetic cataractogenesis.[7, 9] In addition, calpain II has recently been shown to induce cataractogenesis and cleave crystallins in the lenses of a variety of other mammalian species, namely, mice and guinea pigs,[10, 11] calves,[12] monkeys and rabbits.[13]

Calpain II in human lenses has been shown to be the major calpain in the epithelial cells of human lenses showing age-related cortical cataract.[14] Furthermore, using cultured human lenses, it has been shown that an increase in Ca^{2+} levels in these lenses, within physiological limits, results in cortical opacification accompanied by a significant loss of crystallins from the soluble fraction of these lenses.[2] Moreover, analysis of the insoluble fraction from these

lenses reveals high levels of proteolysed vimentin. This cytoskeletal protein is a lens substrate of calpain II and the proteolysis of it is taken as a biomarker of enzyme activation.[8] Taken overall, these results strongly suggest that calpain II plays at least a role in some human cataract.

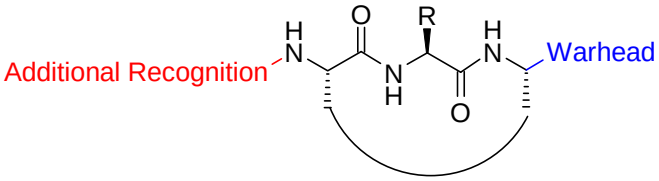
5.2: Use of molecular modelling to design macrocyclic calpain inhibitors

As eluded to in many sections of this thesis the chemical structure of a calpain inhibitor consists of three distinct segments (**Figure 5.1**).

1. A central β-strand backbone or template which is capable of forming the required hydrogen bonds to the enzyme.
2. Two address regions specific for calpain recognition. A wide variety of groups can be employed as address region 1 but usually a rather hydrophobic aromatic group is used. Typically address region 2 is an electrophilic centre which undergoes nucleophilic attack by the cysteine sulfur. The most commonly utilised functional group is an aldehyde.

Figure 5.1: Generic calpain inhibitor

The macrocyclic β-strand templates, of generic structure **4.1**, outlined in **Chapter 4** were designed using these principles. The challenge in this chapter was to design additional recognition and warhead moieties, using molecular modelling, in order to confer potent calpain inhibition to the macrocyclic β-strand templates synthesised in **Chapter 4**.



4.1

The SP/XP docking protocol, described in **Section 2.2,** used to model the dipeptides synthesised in **Chapter 2** is not suitable for modelling the macrocycles contained in the virtual library. The reason for this is that the tripeptide macrocycles extend further into the calpain binding pocket than the dipeptides do. As such the macrocycles interact with residues in the P3/4 binding pocket of the enzyme. As shown in **Figure 5.2** using the "rigid" SP/XP protocol, the Lys_{347} residue blocks the binding pocket around the P3/4 binding region. The result of this is that docking macrocycles using the SP/XP protocol results in these being predicted to be extremely poor calpain inhibitors. The reason for this is that the *N*-substituent of the macrocycle sterically clashes with Lys_{347}.

It is, however, well known that the active site geometry of a protein complex depends heavily upon conformational changes induced by the bound ligand. For this reason Schrödinger's induced fit protocol was used to dock the macrocycles.

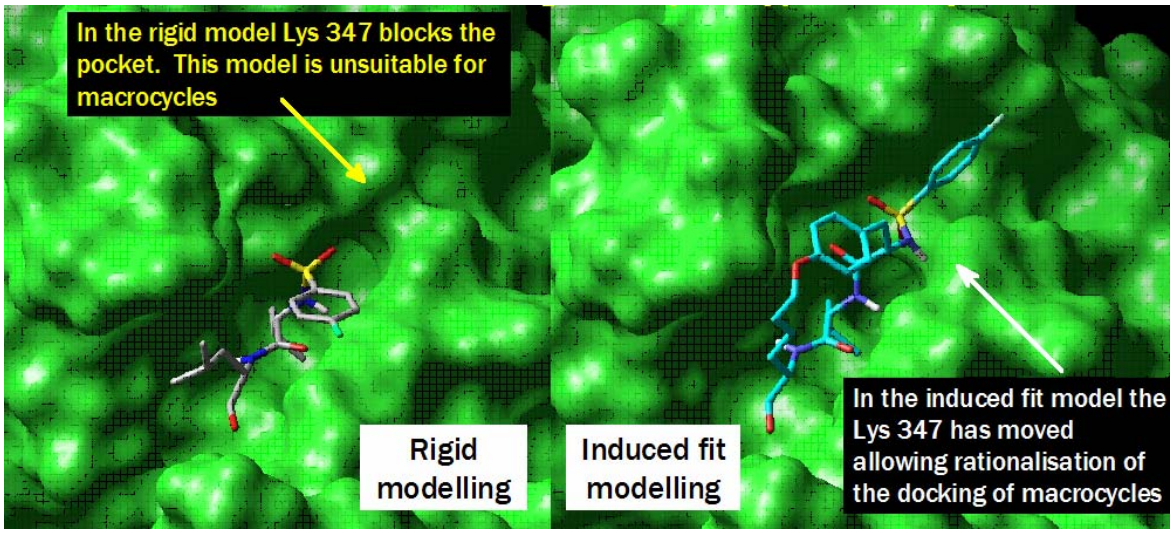


Figure 5.2: Comparison of SP/XP docking and induced fit docking

The induced fit docking protocol solved the steric clash problem encountered during rigid docking by employing a combination of Glide and Prime to exhaustively consider possible binding modes and the associated conformational changes within the enzyme active site. As described in **Section 4.3** MacroModel 9.0 was used to run conformational searches on the macrocycles in order to generate an ensemble of low energy conformers. The lowest energy β-strand conformer was then used as the starting conformation for the induced fit docking.

Induced fit docking was performed using noncharged amino acids Cys_{115} and His_{272}. The centroid of Cys_{115}, Gly_{208}, Gly_{271}, Glu_{349}, and Asn_{253} was chosen for the docking grid generation, as this defines the centre of the active site; the induced fit protocol selects the size of the box by default.

The starting conformer was then docked into the enzyme using Glide. A reduced van der Waals radii of the ligand and protein atoms (0.5) was used in order to generate a diverse ensemble of ligand poses. The side chain of Lys_{347} was removed for the docking and the twenty best poses of this initial docking were kept.

For each ligand pose all residues of the protein within a 5Å distance to the ligand pose were reoriented with PRIME 1.2, including Lys_{347}, to accommodate the ligand. These residues, and the ligand, were then minimised. The ligands were re-docked (with a van der Waals radius of 0.8) to the newly generated protein structure if this was within 30 kcal/mol of the lowest energy protein structure. For each of these protein structures, one ligand pose was kept for evaluation.

Figure 5.3 illustrates the power of the combination of induced fit docking and the use of RCM to prepare macrocycles that are conformationally constrained into a β-strand conformation. This shows the superimposition of the enzyme bound structure of **2.12** and

one of the best (280 nM against calpain II) macrocyclic constraints (**5.10**). From this it is clear that the backbone of the macrocycle is forced to adopt the 'bio-active' β-strand conformation exactly mimicking that of **2.12**.

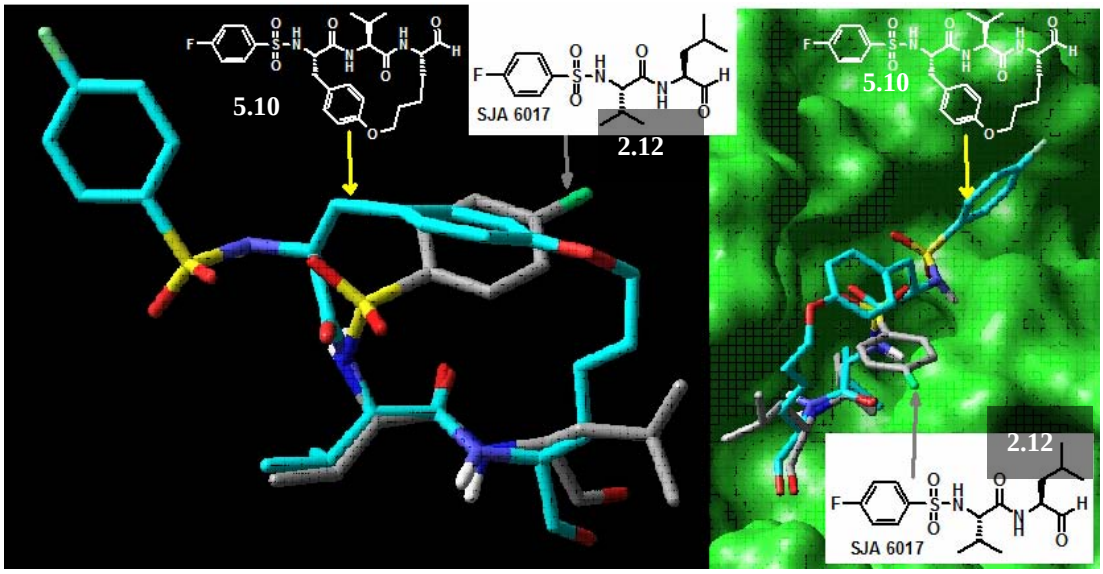


Figure 5.3: Superimposition of **2.12** and **5.10** when bound in the enzyme

5.3: Physicochemical analysis of the *in-silico* library

The successful design of a calpain inhibitor as an anti-cataract agent not only requires the inhibitor to be an excellent β-strand mimic, but it must also be able to diffuse to the site of action (the lens) in sufficient concentration. As such it was also necessary to consider the physicochemical properties of the calpain inhibitor.

The two most important physicochemical properties for eye drugs are aqueous solubility and LogP. The latter is defined as the partition coefficient between water and n-octanol. Within our research group there were two potential administration routes for any anti-cataract agent available to us;

- Topical administration (as either an eye drop or paste)
- Intravitreal injection

Topical administration

Topical drug delivery is the most common treatment for diseases of the anterior segment of the eye. However, this administration route is often severely limited by the low permeability of the cornea, rapid clearance by tear drainage, and absorption into the conjunctiva. Hence, the bioavailability of topically administered drugs is very low.[15] Drug transport processes, and methods to improve bioavailability, have been studied extensively over the past decades.[16-18] A mathematical model of solute transient diffusion across the cornea to the anterior chamber of the eye for topical drug delivery was reported in 2004.[15] As shown in **Figure 5.5** the C_{max} (the maximum solute concentration in the anterior chamber), t_{max} (the time needed to reach C_{max}), and M_a (the fractional amount of solute diffusing into the chamber) are related to LogP.

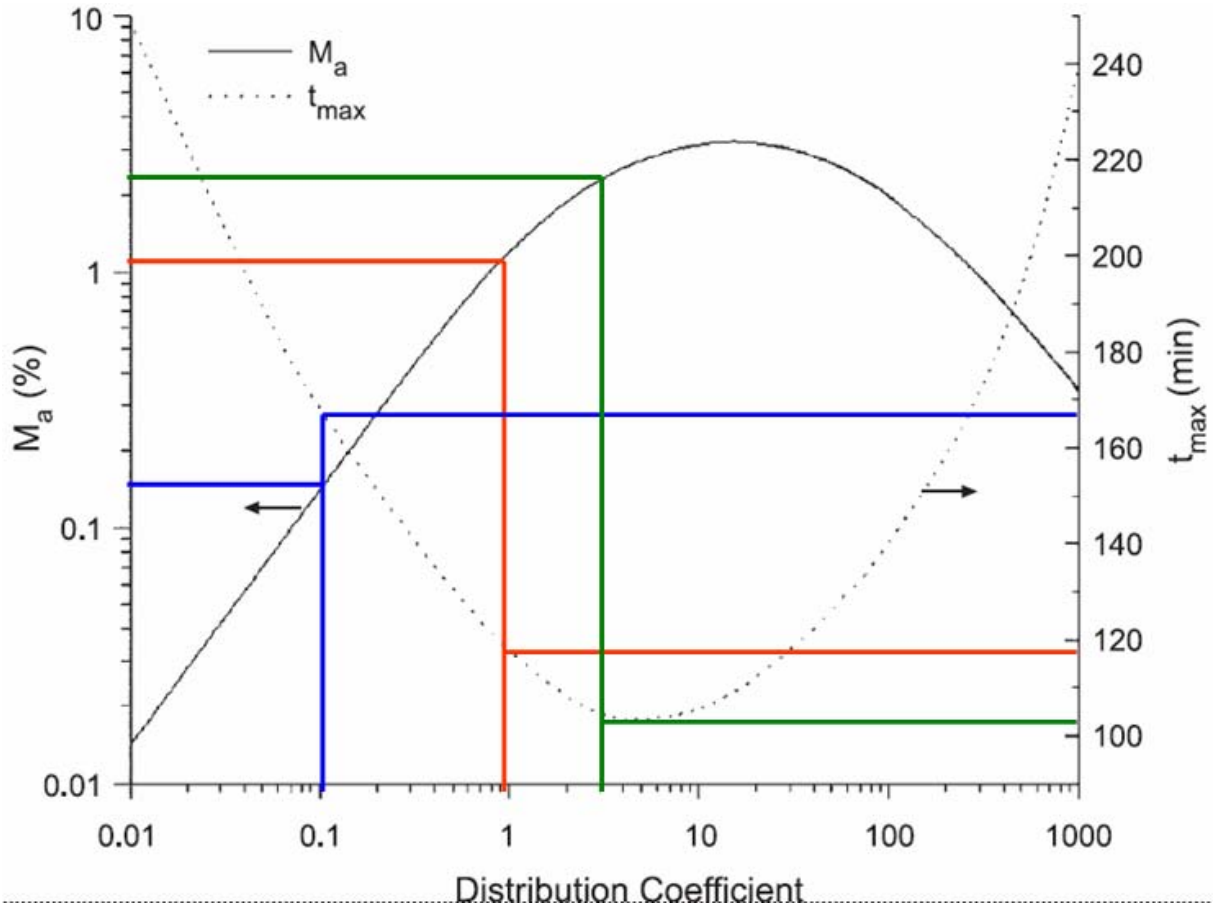
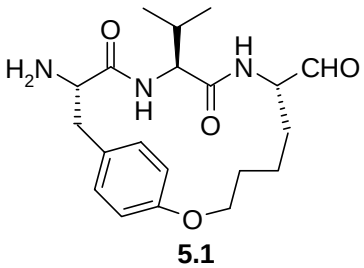


Figure 5.5: mathematical model to describe the interationships between LogP and C_{max}, t_{max} and M_a

From **Figure 5.5** it can be seen that a LogP of between one and fifty is required to achieve greater than one per cent cornea permeability within two hours. It is also well recognised that aqueous solubility, in a given series of very closely related compounds, is generally inversely proportional to LogP. It was decided that all topical agents synthesised should have a LogP between one and three (red and green lines in **Figure 5.5**). While compounds with a LogP greater than three are likely to possess good cornea permeability they are also extremely likely to have negligible aqueous solubility. As such LogP criteria were used in selecting which macrocycles to synthesise. Partition coefficients were calculated (cLogP) using a physicochemical property calculator at www.logp.com.

A number of moieties were proposed, from molecular modeling, to be suitable address regions and as such cLogP determination was required on a large number of compounds. For example, if ten address regions were of interest, and one hundred macrocycles were also of interest, a total of one thousand cLogP calculations were required. To make this process more efficient an approximate cLogP was calculated using a fragment based approach. The cLogP of the macrocyclic aldehyde, amine (**5.1**) was calculated and then the appropriate value for the address region was either added or subtracted (**Table 5.1**).



5.1

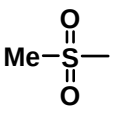
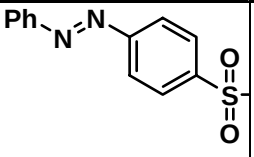
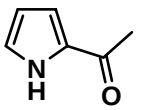
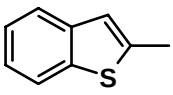
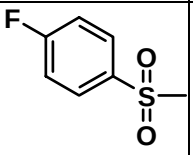
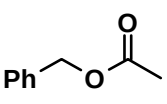
Address moiety	Fragment cLogP	Address moiety	Fragment cLogP
	-1.10		+3.14
	-0.97		+1.32
	-0.88		+0.68

Table 5.1: Fragment based cLogP

Intravitreal administration

As described in the previous **topical administration Section** only a tiny percentage of the drug applied topically on the surface of the eye reaches the internal ocular tissues. Intravitreal injection provides a direct approach for administration of eye drugs to avoid this problem. An intravitreal injection involves the direct injection of a drug solution into the vitreous humor of the eye (**Figure 5.6**). Recently, intravitreal injection of polymeric delivery systems for ganciclovir have been used for the treatment of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome.[19-22] Furthermore, a variety of steroids and antibiotics have long been applied clinically by intravitreal injection.[23]

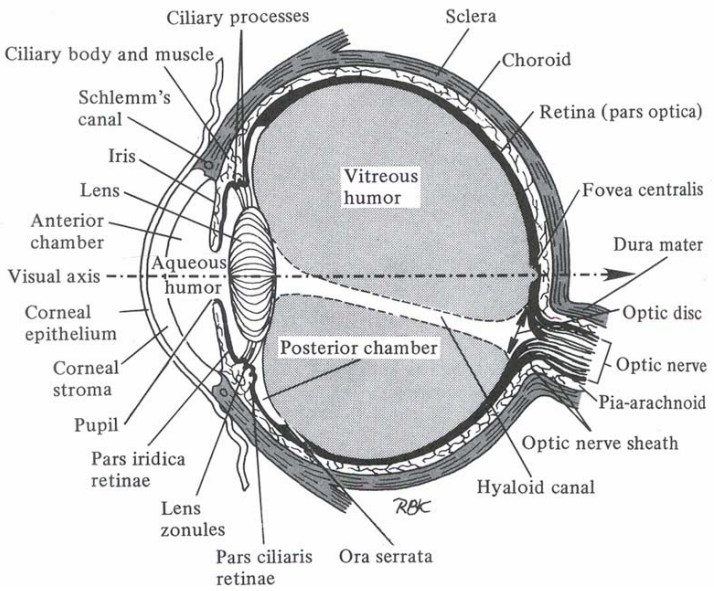
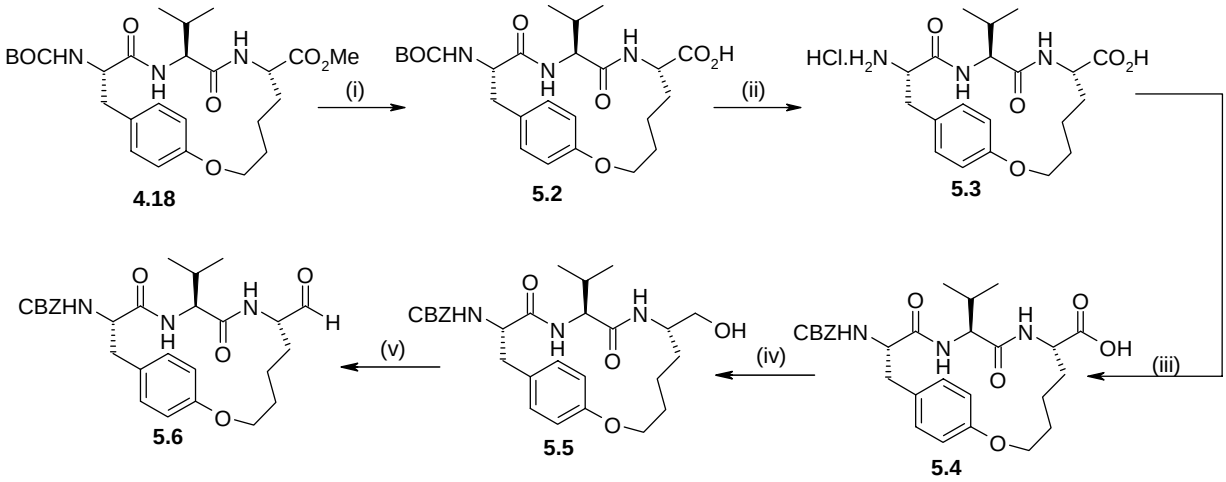


Figure 5.6: The human eye

We considered that the major obstacle for the successful implementation of an intravitreal administration strategy was the very low aqueous solubility of our inhibitors. However, it was postulated that some of the macrocycles in the *in-silico* library which had very low cLogP's would also possess at least moderate aqueous solubility. As such it was believed that intravitreal injection would provide a route of administration for potent compounds which had low cLogP's and hence were not suitable for topical administration (see **Chapter 6**).

5.4: Synthesis of 17-membered ring Tyr-xx-Gly macrocyclic calpain inhibitors

As shown in **Schemes 5.1** and **5.2** a range of macrocycles incorporating different amino acids in the P2 position were synthesised. The aldehyde of both the *N*-CBZ and *N*-4-fluoro-benzene sulfonamide of each P2 variant were prepared. **Scheme 5.1** shows the initial method developed by Matthew Jones in our group to achieve this. Orthogonally protected macrocycle **4.18** was hydrolysed using sodium hydroxide to give carboxylic acid **5.2**. BOC cleavage using 1M aqueous hydrochloric acid gave the fully deprotected amino acid macrocycle **5.3**. The benzyloxycarbonyl group was introduced using benzylchloroformate and weakly basic aqueous reaction conditions to give *N*-CBZ carboxylic acid macrocycle **5.4**. The mixed anhydride of this with ethyl chloroformate was prepared using standard procedures, and subsequent sodium borohydride reduction afforded alcohol **5.5**. A sulfur trioxide/pyridine oxidation gave the required *N*-CBZ P2 valine macrocyclic aldehyde **5.6**. The overall yield from the orthogonally protected macrocycle **4.18** was only 0.8%.

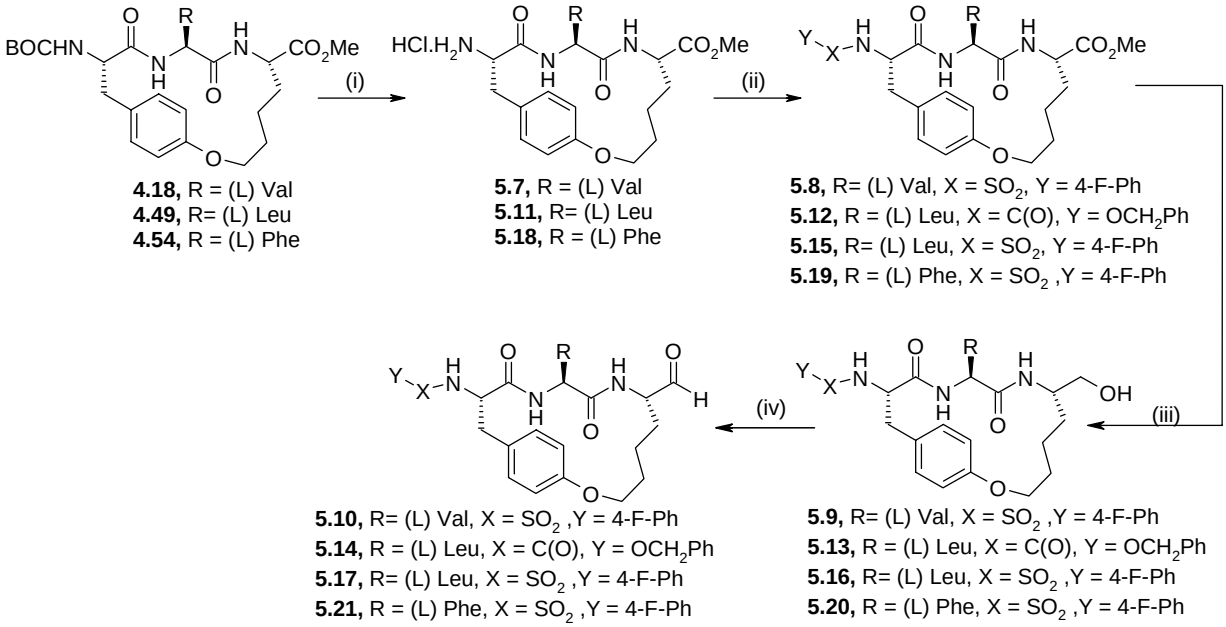


Scheme 5.1. *Reagents and Conditions:* **(i)** NaOH, THF, H_2O, (93%); **(ii)** 1M $HCl_{(aq)}$, (100%); **(iii)** benzyl chloroformate, saturated $NaHCO_{3(aq)}$, (34%); **(iv)** a) ethyl chloroformate, Et_3N, THF; b) $NaBH_4$, THF, (13%); **(v)** SO_3.Pyr, DIPEA, DMSO, DCM, (21%)

This thesis describes the development and use of an alternative higher yielding synthetic route for the preparation of analogues of **5.6 (Scheme 5.2)**. The overall yield from the orthogonally protected macrocycle for the synthesis of the direct leucine analogue of **5.6**

using this methodology was 15%. This is almost a twenty fold improvement compared to the 0.8% achieved using the method in **Scheme 5.1.**

The synthetic methodology used to prepare the P2 variants of **5.6** is shown in **Scheme 5.2**. The *N*-BOC protecting group was cleaved using a solution of 4M hydrogen chloride, in 1,4-dioxane, to give the hydrogen chloride salts of macrocyclic amines **5.7, 5.11** and **5.18**. These were then coupled to either 4-fluorobenzenesulfonyl chloride or benzyl chloroformate using standard reaction conditions. The macrocyclic methyl esters (**5.8, 5.12, 5.15** and **5.19**) were then reduced to the corresponding aldehyde. This was achieved either directly (**5.8** and **5.19**) using DIBAL-H or by a two step process (**5.12** and **5.15**) whereby the ester was reduced using lithium aluminium hydride to an alcohol which was then reoxidised to the aldehyde using a sulfur trioxide pyridine complex. Reduction of phenylalanine methyl ester **5.19**, with DIBAL-H, gave a complex mixture of products by proton NMR. Aldehyde **5.21** was successfully synthesised by Joanna Duncan and as such is not discussed further in this thesis.



Scheme 5.2. *Reagents and Conditions:* **(i)** 4M HCl, 1,4-dioxane, (all 100%);

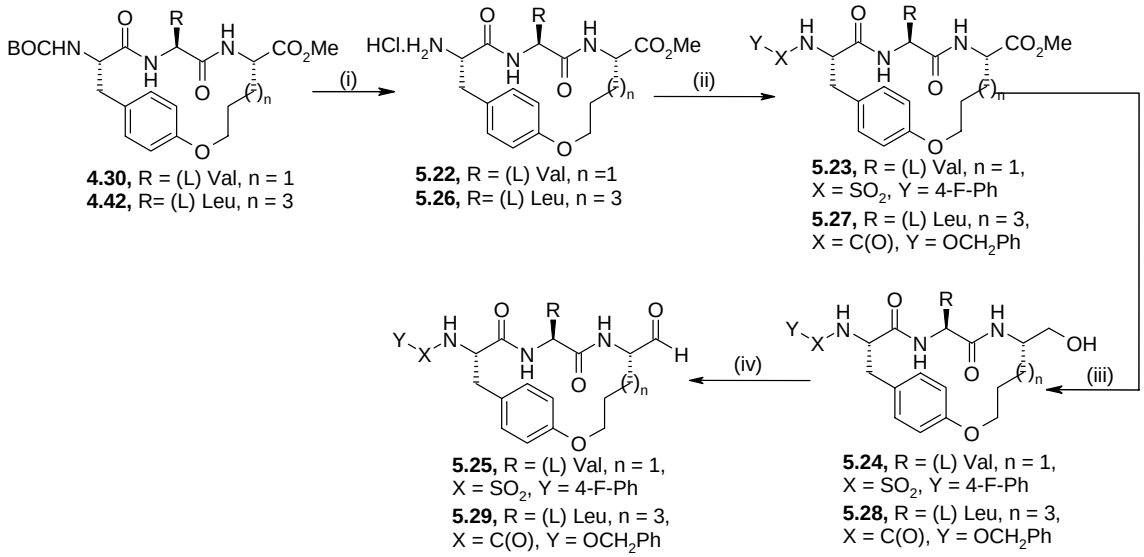
For R= Val, X = SO_2, Y = 4-F-Ph; (ii) 4-fluorobenzenesulfonyl chloride, DIPEA, DMF, (36%), **(iii)** DIBAL-H, DCM, (4% **5.9** and 17% **5.10**)

For R=Leu, X = C(O), Y = OCH_2Ph; (ii) benzyl chloroformate, DIPEA, DMF, (43%); **(iii)** $LiAlH_4$, THF, (83%); **(iv)** SO_3.Pyr, DIPEA, DMSO, DCM, (42%);

For R=Leu, X = SO_2, Y = 4-F-Ph; (ii) 4-fluorobenzenesulfonyl chloride, DIPEA, DMF, (7%); **(iii)** $LiAlH_4$, THF, (87%); **(iv)** SO_3.Pyr, DIPEA, DMSO, DCM, (69%);
For R=Phe, X = SO_2, Y = 4-F-Ph; (ii) 4-fluorobenzenesulfonyl chloride, DIPEA, DMF, (18%), **(iii)** DIBAL-H, DCM, (0%)

5.5: Synthesis of 16 and 18-membered Tyr-xx-Gly macrocyclic calpain inhibitors

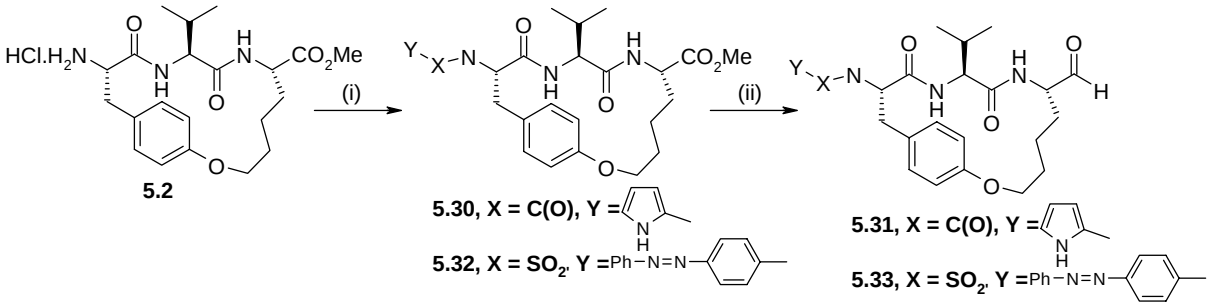
As shown in **Scheme 5.3** the synthesis of the Tyr-xx-Gly based sixteen and eighteen membered macrocyclic calpain inhibitors was achieved. The sixteen membered macrocyclic *N*-4-fluorobenzene sulfonamide (**5.25**) was synthesised from orthogonally protected macrocycle **4.30**. The *N*-BOC protecting group of **4.30** was cleaved with 4M hydrogen chloride in 1,4-dioxane to give the hydrogen chloride salt of amine **5.22**. The sulfonamide **5.23** was prepared by coupling amine **5.22** to 4-fluorobenzenesulfonyl chloride under standard conditions. Reduction of methyl ester **5.23** with DIBAL-H gave a mixture of both the alcohol **5.24** and the desired aldehyde **5.25**. These were separated by flash chromatography. The eighteen membered *N*-CBZ macrocycle (**5.29**) was synthesised from orthogonally protected macrocycle **4.42**. The *N*-BOC protecting group of **4.42** was removed by treatment with 4M hydrogen chloride in 1,4-dioxane. The resultant hydrogen chloride salt of amine **5.26** was reacted with benzylchloroformate to give *N*-CBZ macrocycle **5.27**. Reduction with lithium aluminium hydride gave alcohol **5.28** and subsequent oxidation, with sulfur trioxide pyridine complex gave aldehyde **5.29**.



Scheme 5.3. *Reagents and Conditions:* **For R = Val, n = 1; (i)** 4M HCl, 1,4-dioxane, (100%); **(ii)** 4-fluorobenzenesulfonyl chloride, DIPEA, DMF, (16%); **(iii)** DIBAL-H, DCM, (7% **5.24** and 5% **5.25**); **For R = Leu, n = 3; (i)** 4M HCl, 1,4-dioxane (100%); **(ii)** benzyl chloroformate, DIPEA, DMF, (69%); **(iii)** $LiAlH_4$, THF, (72%); **(iv)** SO_3.Pyr, DIPEA, DMSO, DCM, (82%)

5.6: Synthesis of various *N*-substituted 17-membered Tyr-xx-Gly macrocycles

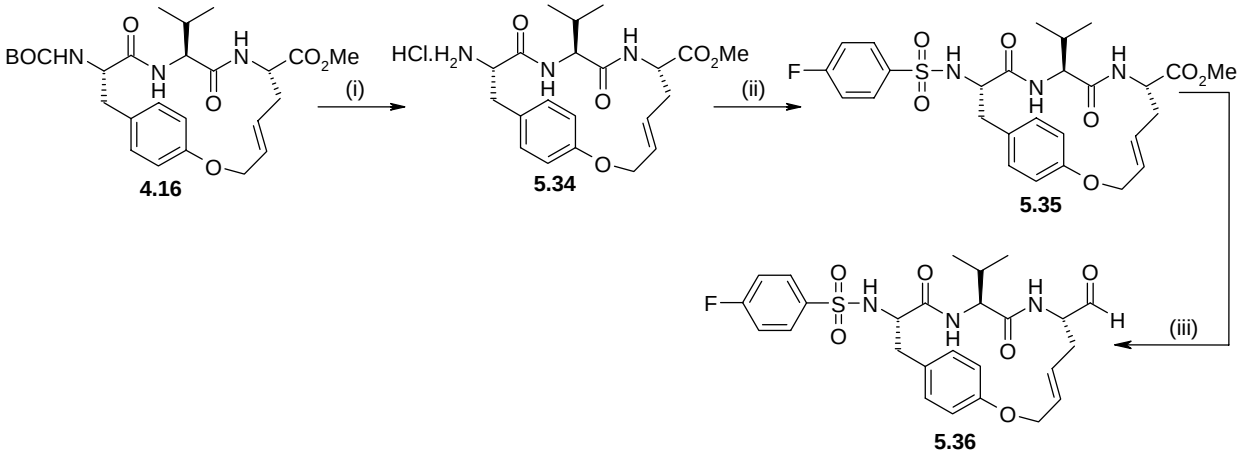
The synthesis of further address region analogues of **5.6** in order to generate SAR for the address region of the macrocycles was carried out by Matthew Jones (**Scheme 5.4**).



Scheme 5.4. *Reagents and Conditions:* **For X = C(O) and Y = 2-pyrrole (i)** EDC, $HOBt.H_2O$, DIPEA, Pyrrole-2-carboxylic acid, DMF, (21%); **(ii)** DIBAL-H, DCM, (33%). **For X = SO_2 and Y = 4-(phenyldiazo) benzene (i)** 4-azabenzenephenyl sulfonyl chloride, DIPEA, DMF, (30%); **(ii)** DIBAL-H, DCM, (6%)

5.7: Synthesis of unsaturated 17-membered Tyr-Val-Gly macrocycle

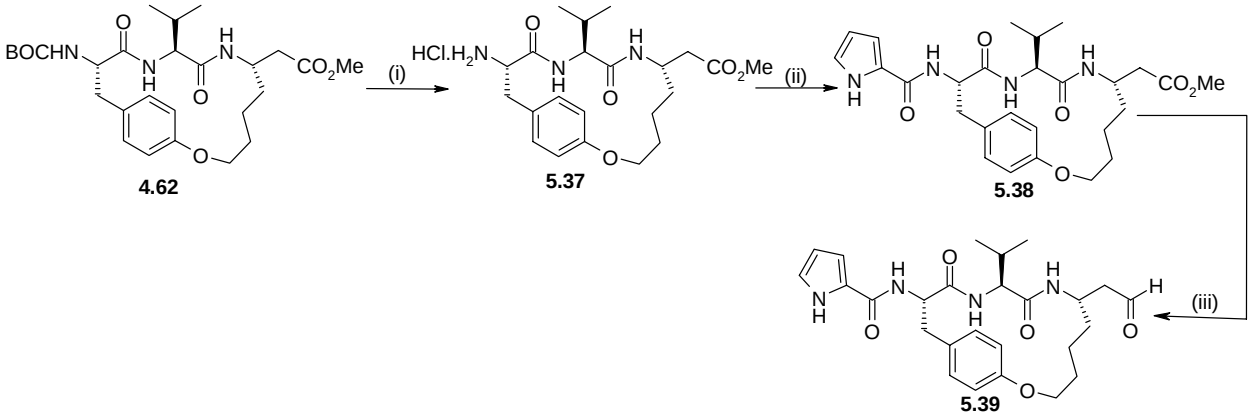
The synthesis of an unsaturated calpain inhibitor is shown in **Scheme 5.5**. Compound **5.36** was selected for synthesis to investigate the effect on calpain inhibition of incorporating a double bond into a macrocyclic calpain inhibitor. The *N*-BOC protecting group of orthogonally protected macrocycle **4.16** was cleaved on treatment with 4M hydrogen chloride in 1,4-dioxane to give the hydrogen chloride salt of amine **5.34**. *N*-4-fluorobenzene sulfonamide **5.35** was prepared by reaction of amine **5.34**. Reduction of ester **5.35** with DIBAL-H gave aldehyde **5.36**.



Scheme 5.5. *Reagents and Conditions:* **(i)** 4M HCl, 1,4-dioxane, (100%); **(ii)** 4-fluoro-benzenesulfonyl chloride, DIPEA, DMF, (33%); **(iii)** DIBAL-H, DCM, (18%)

5.8: Synthesis of 17-membered ring Tyr-Val-Gly β-amino acid macrocycle

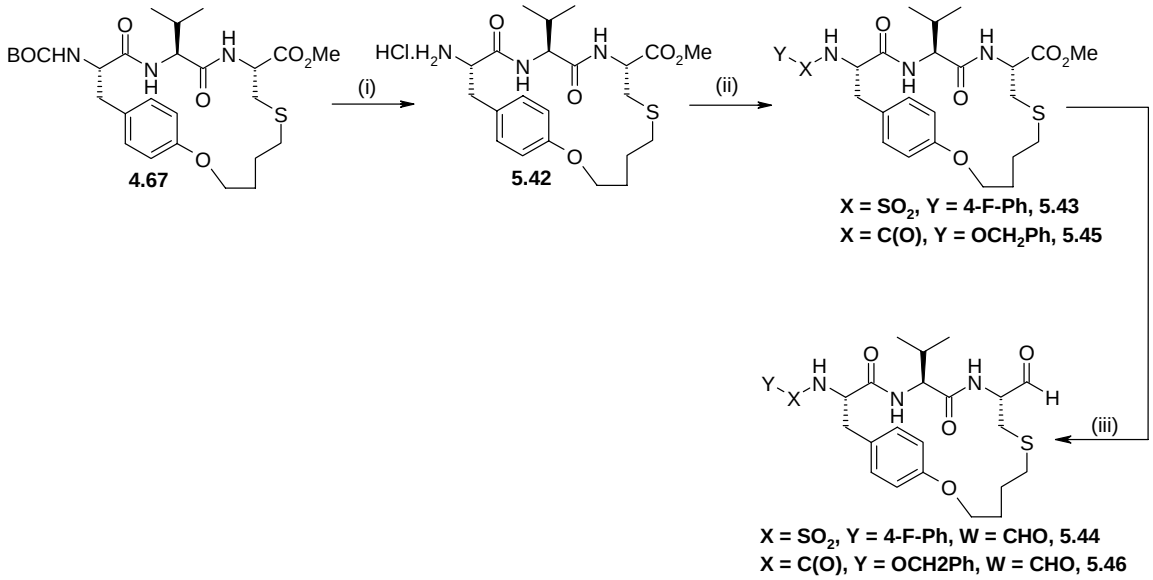
As shown in **Scheme 5.6** the synthesis a β-amino acid warhead macrocycle was achieved in our lab by Matthew Jones. The *N*-BOC protecting group of orthogonally protected macrocycle **4.62** was cleaved on treatment with saturated ethereal hydrogen chloride. The hydrogen chloride salt of amine **5.37** was coupled with pyrrole-2-carboxylic acid and methyl ester **5.38** was reduced using DIBAL-H to give aldehyde **5.39**.



Scheme 5.6. *Reagents and Conditions:* **(i)** $HCl_{(g)}$, Et_2O, (100%); **(ii)** EDC, HOBt.H_2O, DIPEA, Pyrrole-2-carboxylic acid, DMF, (21%); **(iii)** DIBAL-H, DCM, (2%)

5.9: Synthesis of 19-membered ring Tyr-Val-Cys macrocyclic calpain inhibitors

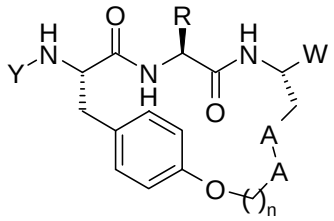
Molecular modelling indicates that cysteine P1 substituted Tyr-xx-Cys macrocycles are excellent β-strand mimics (see **Chapter 4**). As such macrocycle **4.67** was elaborated into a number of calpain inhibitors (**Scheme 5.7**). The *N*-BOC protecting group of orthogonally protected macrocycle **4.67** was cleaved with a solution of 4M hydrogen chloride in 1,4-dioxane to give the hydrogen chloride salt of amine **5.42**. The preparation of the *N*-CBZ analogue is described in this thesis whilst the *N*-4-fluorobenzene sulfonamide analogue was prepared by another member of the group (Matthew Jones). The *N*-CBZ methyl ester (**5.45**) was prepared by reacting amine **5.42** with benzyloxycarbonyl chloroformate. Reduction of methyl ester **5.45** with DIBAL-H gave aldehyde **5.46**.



Scheme 5.7. *Reagents and Conditions:* **For X = SO_2, Y = 4-F-Ph (i)** 4M HCl, 1,4-dioxane, (100%); **(ii)** 4-fluorobenzenesulfonyl chloride, DIPEA, DMF, (17%); **(iii)** DIBAL-H, DCM, (12%). **For X = C(O), Y = OCH_2Ph (i)** 4M HCl, 1,4-dioxane, (100%); **(ii)** benzyl chloroformate, DIPEA, DMF, (18%); **(iii)** DIBAL-H, DCM, (17%)

5.10: Calpain inhibition results of the macrocyclic β-strand mimics

A summary of calpain inhibition assay results for the Tyr-xx-Gly series of macrocyclic β-strand mimics is shown in **Table 5.2**. See **appendix 1** for details of the calpain inhibition assay.



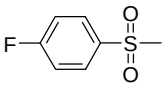
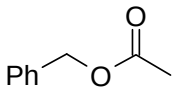
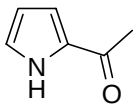
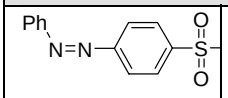
Y	R	W	n	A	Cpmd #	Induced fit Glide Score	% conformation (Boltzmann weighted) β-strand	β-twist	Warhead distance to Cys (Å)	IC_{50} (nM)	cLogP
	Val	CH_2OH	0	CH_2	**5.24**	-8.80	99.54	0.41	>>5	6490	-0.86
	Val	CH_2OH	1	CH_2	**5.9**	-9.80	99.60	0	4.80	1090	-0.61
	Leu	CH_2OH	1	CH_2	**5.16**	-10.4	100	0	3.80	930	-0.53
	Val	CHO	0	CH_2	**5.25**	-10.2	100	0	3.20	3710	-0.68
	Val	CHO	1	CH_2	**5.10**	-9.37	81.16	9.36	4.33	280	-0.20
	Val	CHO	1	CH	**5.36**	-7.72	32.60	6.02	>5	2860	-0.75
	Leu	CHO	1	CH_2	**5.17**	-9.58	76.05	23.95	4.06	45	-0.37
	Leu	CH_2OH	1	CH_2	**5.13**	-10.2	100	0	4.30	704	1.27
	Leu	CH_2OH	2	CH_2	**5.28**	-8.90	89.42	5.12	4.30	1100	1.47
	Val	CHO	1	CH_2	**5.6**	9.09	50.62	57.86	3.63	85	1.33
	Leu	CHO	1	CH_2	**5.14**	-8.60	50.62	47.86	3.70	27	1.59
	Leu	CHO	2	CH_2	**5.29**	-10.3	89.88	9.54	3.50	180	1.80
	Val	CHO	1	CH_2	**5.31**	-8.42	78.82	8.52	3.89	690	0.40
	Val	CH_2CHO	1	CH_2	**5.39**	-9.50	81.55	7.38	3.90	75230	1.91
$MeSO_2$	Val	CHO	1	CH_2	**5.40**	-9.50	86.48	1.43	3.50	949	1.70
	Val	CH_2CHO	1	CH_2	**5.41**	-9.00	95.41	0.17	>>5	112800	-0.73
	Val	CHO	1	CH_2	**5.33**	-6.22	84.49	4.55	4.07	1530	3.28

Table 5.2: Calpain inhibition assay results for the Tyr-xx-Gly series

From the assay results reported in **Table 5.2** a number of SAR conclusions can be drawn;

Effect of P2 amino acid on calpain inhibition

Leucine is the best P2 substituent. In all cases where the direct valine and leucine analogues were prepared, leucine results in more potent calpain inhibition than valine. Examples include the direct analogues *N*-CBZ leucine aldehyde **5.14** (27 nM) / *N*-CBZ valine aldehyde **5.6** (85 nM) and N-4-F-benzene sulfonamide leucine aldehyde **5.17** (45 nM) / *N*-4-F-benzene sulfonamide valine aldehyde **5.10** (280 nM).

There is no single molecular modelling parameter that can accurately rationalise this effect. It is simply not possible to predict the most potent calpain inhibitor within a series of very closely related analogues. It is instead a complex relationship between percentage β-strand conformation, warhead distance from the active site Cys and induced fit docking score. For example comparing aldehydes **5.10** (280 nM) and **5.17** (45 nM) there is approximately a six fold difference in calpain inhibition. Molecular modelling data shows that **5.17** has the better induced fit docking score (-9.58 and -9.37 respectively) and warhead distance to Cys_{105} (4.06 Å compared to 4.33 Å) but a lower percentage of β-strand conformation (76.05% to 81.16%).

Effect of removing the electrophilic warhead

If an excellent β-strand mimic is obtained which possesses high binding affinity for calpain an electrophilic warhead group is not required for potent inhibition. Alcohols **5.9** (1090 nM)**, 5.13** (704 nM) and **5.16** (930 nM) are all potent calpain inhibitors with no reactive electrophilic warhead. The best compound (**5.13**) is to the best of our knowledge the most potent non-warhead active site binding calpain inhibitor known.

Effect of macrocyclic ring size on calpain inhibition

A seventeen membered macrocycle is optimal for calpain inhibition in the Tyr-xx-Gly macrocyclic series. This is concluded from SAR studies across four compounds. The seventeen membered *N*-4-fluorobenzenesulfonamide macrocyclic aldehyde **5.10** (280 nM) is over thirteen fold potent than the direct sixteen membered analogue **5.25** (3710 nM). The seventeen membered *N*-CBZ macrocyclic aldehyde **5.14** (27 nM) is approximately 6.5 fold more potent as a calpain inhibitor than the direct eighteen membered analogue **5.19** (180 nM).

These findings are also confirmed by the alcohols of the macrocycles. These show the same results. The seventeen membered ring alcohol **5.13** (704 nM) is better than the eighteen membered ring alcohol **5.28** (1100 nM) which is in turn better than the sixteen membered ring alcohol **5.24** (6490 nM).

Effect of address region on calpain inhibition

The incorporation of different address region moieties onto the same macrocyclic β-strand template has a very significant effect on calpain inhibition. For example there is a six fold decrease in calpain inhibition by changing the *N*-substituent from *N*-4-fluorobenzene sulfonamide **5.10** (280 nM) to 4-(phenyldiazo) benzene sulfonamide **5.33** (1530 nM). The effect is rationalised using molecular modelling. Aldehyde **5.10** has a much higher induced fit docking score than **5.33** (-9.37 and -6.22 respectively)

It can also be concluded that benzyloxycarbonyl is an excellent address moiety for calpain inhibition. Incorporation of this affords the most potent calpain inhibitor **5.14** (27 nM). Molecular modelling indicates that this is because *N*-CBZ confers the best balance of percentage β-strand conformation, warhead to Cys_{105} distance and induced fit docking score.

Effect of incorporating unsaturation on calpain inhibition

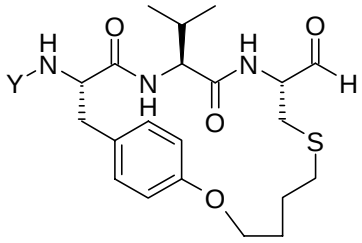
Retention of the *E* double bond from the RCM reaction is detrimental for calpain inhibition. The unsaturated macrocycle **5.36** (2860 nM) is ten fold less potent than the direct saturated analogue **5.10** (280 nM). Molecular modelling clearly indicates that **5.36** does not exist in a high percentage β-strand conformation compared to **5.10** (32.60% compared to 81.16%). In addition, the electrophilic warhead of unsaturated macrocycle **5.36** is much further away from the active site cysteine (> 5 Å) than the electrophilic warhead is in **5.10.**

Effect of using a β-amino acid warhead

The use a β-amino acid warhead eliminates virtually all calpain inhibitory activity. The IC_{50} values of the β-amino acid analogues **5.39** (75230 nM) and **5.41** (112800 nM) are over two orders of magnitude greater than those of the corresponding α-amino acid analogues **5.31** (690 nM) and **5.40** (949 nM).

Calpain inhibition results of the Tyr-Val-Cys based macrocycles

A summary of calpain inhibition assay results for the Tyr-Val-Cys series of macrocyclic β-strand mimics is shown in **Table 5.3**. See **appendix 1** for details of the calpain inhibition assay.



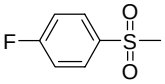
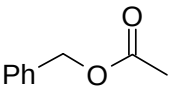
Y	Compound Number	Induced fit Glide Score	% conformation (Boltzmann weighted) β-strand	β-twist	Warhead distance to Cys (Å)	IC_{50} (nM)	cLogP
	**5.44**	-7.50	97.85	1.90	4.00	2400	0.39
	**5.46**	-10.1	93.96	3.88	4.70	295	1.89

Table 5.3: Effect of P1 residue on calpain inhibition

The P1 cysteine based macrocycles **5.44** and **5.46** are both β-strand mimetics as they possess moderate to excellent calpain inhibition (2400 nM and 285nM respectively). They are however less potent than their direct P1 glycine analogues (**5.10**, 280 nM and **5.6**, 85 nM).

The inhibitory activity of the Tyr-Val-Cys based macrocycles are influenced to a greater extent by the *N*-substituent. Changing from *N*-CBZ (**5.6**, 85 nM) to *N*-4-fluorobenzene sulfonamide (**5.10**, 280 nM) in the P1 glycine series results in approximately a three fold decrease in activity whereas in the P1 cysteine series the same substitution (**5.46**, 295 nM and **5.44**, 2400 nM) results in an eight fold decrease in activity. This substituent effect can be

explained using the induced fit docking scores. In the P1 cysteine series molecular modelling indicates that *N*-CBZ macrocycle **5.46** (-10.1) is a much better calpain binder than *N*-4-fluorobenzene sulfonamide macrocycle **5.44** (-7.50). The difference in their induced fit docking scores is 2.6. In the P1 glycine series the difference in the induced fit docking score for the *N*-CBZ (**5.6**, -9.09) and the *N*-4-fluorobenzene sulfonamide (**5.10**, -9.37) analogues is only 0.32.

5.11: Conclusions and future work

A number of calpain inhibitors in two different macrocyclic series were designed using induced fit molecular modelling and *in-silico* physicochemical calculations. Nineteen of these were synthesised and tested. Overall from this work it is concluded that Tyr-xx-Gly based macrocycles are better calpain inhibitors than Tyr-xx-Cys based macrocycles.

In the Tyr-xx-Gly macrocyclic system a number of variables were investigated. The ring size was varied from sixteen to eighteen and it was found that a seventeen membered ring was optimal for calpain inhibition.

The central amino acid of the macrocyclic system was systematically varied and leucine was discovered to result in the best inhibition.

A number of address regions were investigated. Benzyloxycarbonyl was found to result in the most potent calpain inhibition with the best macrocyclic inhibitor (**5.14**) having an IC_{50} against calpain II of 27 nM.

The effects of incorporating unsaturation in the macrocycle and using a β-amino acid aldehyde were also investigated. Both of these approaches were detrimental to calpain inhibition with incorporation of a β-amino acid aldehyde being particularly bad.

One of the most promising findings from this chapter is that macrocyclic inhibitors which are 'pre-organised' into the 'bio-active' β-strand conformation do not require an electrophilic warhead for potent inhibition. The best non-electrophilic warhead macrocycle (**5.13**) had an IC_{50} against calpain II of 704 nM. This finding should motivate extensive further work in this area.

References for Chapter 5

1. Azuma, M.; Fukiage, C.; David, L. L.; Shearer, T. R., Activation of calpain in lens: A review and proposed mechanism. *Experimental Eye Research* **1997,** 64, (4), 529-538.
2. Biswas, S.; Harris, F.; Dennison, S.; Singh, J.; Phoenix, D. A., Calpains: targets of cataract prevention? *Trends in Molecular Medicine* **2004,** 10, (2), 78-84.
3. Biswas, S.; Harris, F.; Dennison, S.; Singh, J. P.; Phoenix, D., Calpains: enzymes of vision? *Medical Science Monitor* **2005,** 11, (9), RA301-RA310.
4. Robertson Lucinda, J. G.; Morton James, D.; Yamaguchi, M.; Bickerstaffe, R.; Shearer Thomas, R.; Azuma, M., Calpain may contribute to hereditary cataract formation in sheep. *Investigative ophthalmology & visual science* **2005,** 46, (12), 4634-40.
5. Andersson, M.; Sjostrand, J.; Karlsson, J. O., Calpains in the human lens: Relations to membranes and possible role in cataract formation. *Ophthalmic Research* **1996,** 28, 51-54.
6. Shearer, T. R.; Ma, H.; Shih, M.; Fukiage, C.; Azuma, M., Calpains in the lens and cataractogenesis. *Methods in molecular biology (Clifton, N.J.)* **2000,** 144, 277-85.
7. Thampi, P.; Hassan, A.; Smith, J. B.; Abraham, E. C., Enhanced C-terminal truncation of alpha A- and alpha B-crystallins, in diabetic lenses. *Investigative Ophthalmology & Visual Science* **2002,** 43, (10), 3265-3272.
8. Goll, D. E.; Thompson, V. F.; Li, H.; Wei, W.; Cong, J., The calpain system. *Physiological Reviews* **2003,** 83, (3), 731-801.
9. Sakamoto-Mizutani, K.; Fukiage, C.; Tamada, Y.; Azuma, M.; Shearer, T. R., Contribution of ubiquitous calpains to cataractogenesis in the spontaneous diabetic WBN/Kob rat. *Experimental Eye Research* **2002,** 75, (5), 611-617.
10. Fukiage, C.; Azuma, M.; Nakamura, Y.; Tamada, Y.; Nakamura, M.; Shearer, T. R., SJA6017, a newly synthesized peptide aldehyde inhibitor of calpain: amelioration of cataract in cultured rat lenses. *Biochimica Et Biophysica Acta-Molecular Basis of Disease* **1997,** 1361, (3), 304-312.
11. Ueda, Y.; Duncan, M. K.; David, L. L., Lens proteomics: The accumulation of crystallin modifications in the mouse lens with age. *Investigative Ophthalmology & Visual Science* **2002,** 43, (1), 205-215.
12. Ueda, Y.; McCormack, A. L.; Shearer, T. R.; David, L. L., Purification and characterization of lens specific calpain (Lp82) from bovine lens. *Experimental Eye Research* **2001,** 73, (5), 625-637.
13. Nakajima, T.; Fukiage, C.; Azuma, M.; Ma, H.; Shearer, T. R., Different expression patterns for ubiquitous calpains and Capn3 splice variants in monkey ocular tissues. *Biochimica Et Biophysica Acta-Gene Structure and Expression* **2001,** 1519, (1-2), 55-64.

14. Andersson, M.; Sjostrand, J.; Andersson, A. K.; Andersen, B.; Karlsson, J. O., Calpains in Lens Epithelium from Patients with Cataract. *Experimental Eye Research* **1994,** 59, (3), 359-364.
15. Zhang, W.; Prausnitz, M. R.; Edwards, A., Model of transient drug diffusion across cornea. *Journal of Controlled Release* **2004,** 99, (2), 241-258.
16. Lee, V. H.; Robinson, J. R., Topical ocular drug delivery: recent developments and future challenges. *J. Ocul. Pharmacol.* **1986,** 2, (1), 67-108.
17. Jarvinen, K.; Jarvinen, T.; Urtti, A., Ocular Absorption Following Topical Delivery. *Advanced Drug Delivery Reviews* **1995,** 16, (1), 3-19.
18. Schoenwald, R. D., *Ophthalmic Drug Delivery Systems*. Marcel Dekker: New York, 2003; p 135– 179.
19. Ussery, F. M.; Gibson, S. R.; Conklin, R. H.; Piot, D. F.; Stool, E. W.; Conklin, A. J., Intravitreal Ganciclovir in the Treatment of Aids-Associated Cytomegalo-Virus Retinitis. *Ophthalmology* **1988,** 95, (5), 640-648.
20. Cochereaumassin, I.; Lehoang, P.; Lautierfrau, M.; Zazoun, L.; Marcel, P.; Robinet, M.; Matheron, S.; Katlama, C.; Gharakhanian, S.; Rozenbaum, W.; Ingrand, D.; Gentilini, M., Efficacy and Tolerance of Intravitreal Ganciclovir in Cytomegalovirus Retinitis in Acquired-Immune-Deficiency-Syndrome. *Ophthalmology* **1991,** 98, (9), 1348-1355.
21. Cantrill, H. L.; Henry, K.; Melroe, N. H.; Knobloch, W. H.; Ramsay, R. C.; Balfour, H. H., Treatment of Cytomegalo-Virus Retinitis with Intravitreal Ganciclovir - Long-Term Results. *Ophthalmology* **1989,** 96, (3), 367-374.
22. Peyman, G. A.; Schulman, J. A., Intravitreal Drug-Therapy. *Japanese Journal of Ophthalmology* **1989,** 33, (4), 392-404.
23. Tojo, K.; Isowaki, A., Pharmacokinetic model for in vivo/in vitro correlation of intravitreal drug delivery. *Advanced Drug Delivery Reviews* **2001,** 52, (1), 17-24.

6.1: Ovine *in-vivo* cataract model

This chapter describes the *in-vivo* evaluation studies performed on three of the best β-strand mimics identified in **Chapters 2** and **5**. It also describes the synthetic optimisation which was required in order to successfully carry out a multi-gram synthesis of each of these.

As described in **Chapter 1**, calpain over activation is thought to be involved in a number of human diseases of which cataract formation is one such example. In order to develop a calpain inhibitor as a human drug, animal trials first need to be conducted in order to demonstrate that the proposed drug is both safe and efficacious. Our collaborators at Lincoln University, New Zealand have developed an animal model for cataract. They have established a flock of sheep which develop hereditary cataracts. This was achieved by mating a cataractous Romney ram with normal-eyed, unrelated Coopworth ewes.[1]

Our colleagues at Lincoln University have published[1] a number of findings which suggest that calpain over-activation is involved in ovine cataract formation and development;

- three calpain isoforms are present in young lamb lenses.
- lens calcium concentration increases in the early stages of ovine cataract formation and this is ten fold higher in lambs with mature cataract than in lambs with normal lenses.
- calpain activity decreases as the cataracts develop

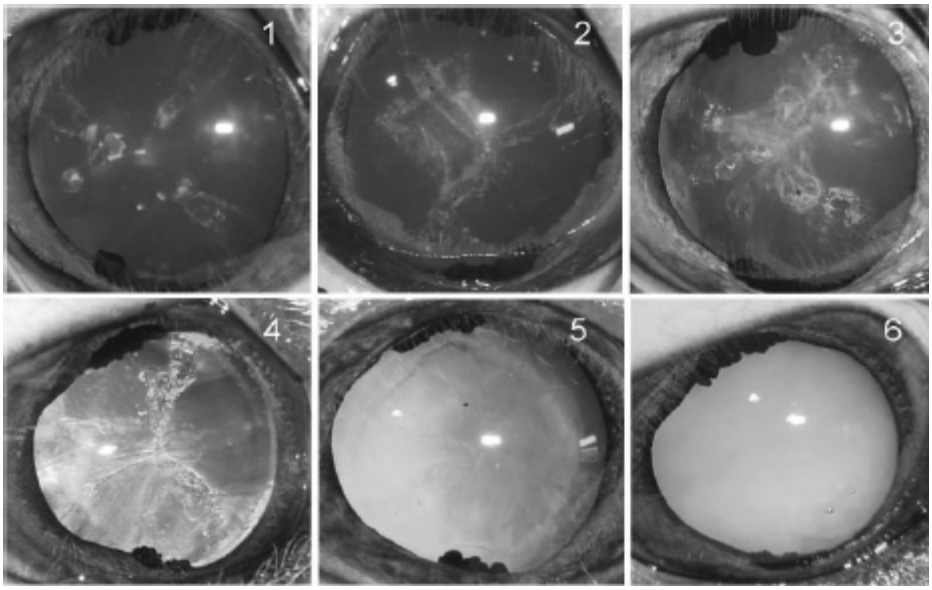
The presence of calpain and calcium elevation during ovine cataract formation strongly suggests that proteolysis plays a key role in opacification of ovine lens. As such this animal model of cataract provides an excellent *in-vivo* model in which to establish efficacy of the best calpain inhibitors described in **Chapters 2** and **5** as anti-cataract agents. In order that

quantification of the *in-vivo* efficacy results could be achieved two scoring systems for cataractogenesis were devised; a one to six scale and a one to seven scale.

One to six scale

Cataract development in the ovine lens is divided into three main stages, and each of these into two sub stages (**Figure 6.1**);

- Early cataracts (stages 1 to 2) consist of discrete anterior and posterior cortical opacities, usually centered at the lens suture lines.
- mid-stage (stages 3 to 4) cataracts form spoke-like patterns radiating to the peripheral cortex and epithelial layer.
- mature cataract involves the whole lens (stages 5 to 6).

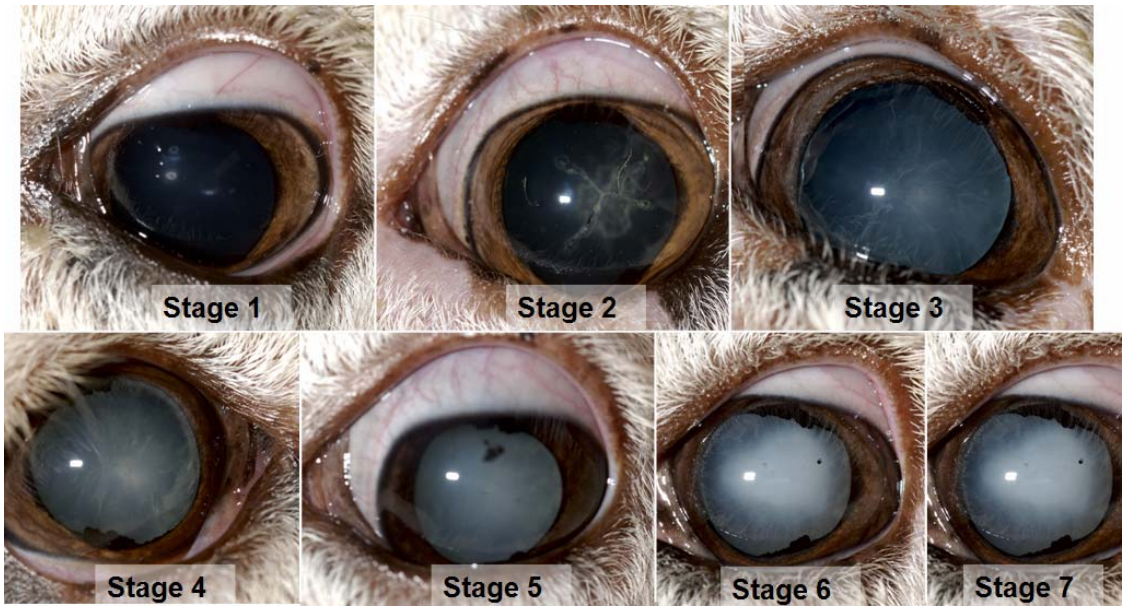


(1) Small opacity detected at either the anterior or posterior suture line. **(2)** Small opacities detected at both suture lines. **(3)** Opacification at sutures and mild cortical involvement. **(4)** Moderate to severe cortical involvement. **(5)** Immature cataract involving the whole lens. **(6)** Mature cataract.

Figure 6.1: One to six cataract scoring system

One to seven scale

The one to seven cataract scale was developed in order to increase the sensitivity of the scale around the midpoint of cataract development. Essentially stage four of the one to six scale has been split into two (**Figure 6.2**).



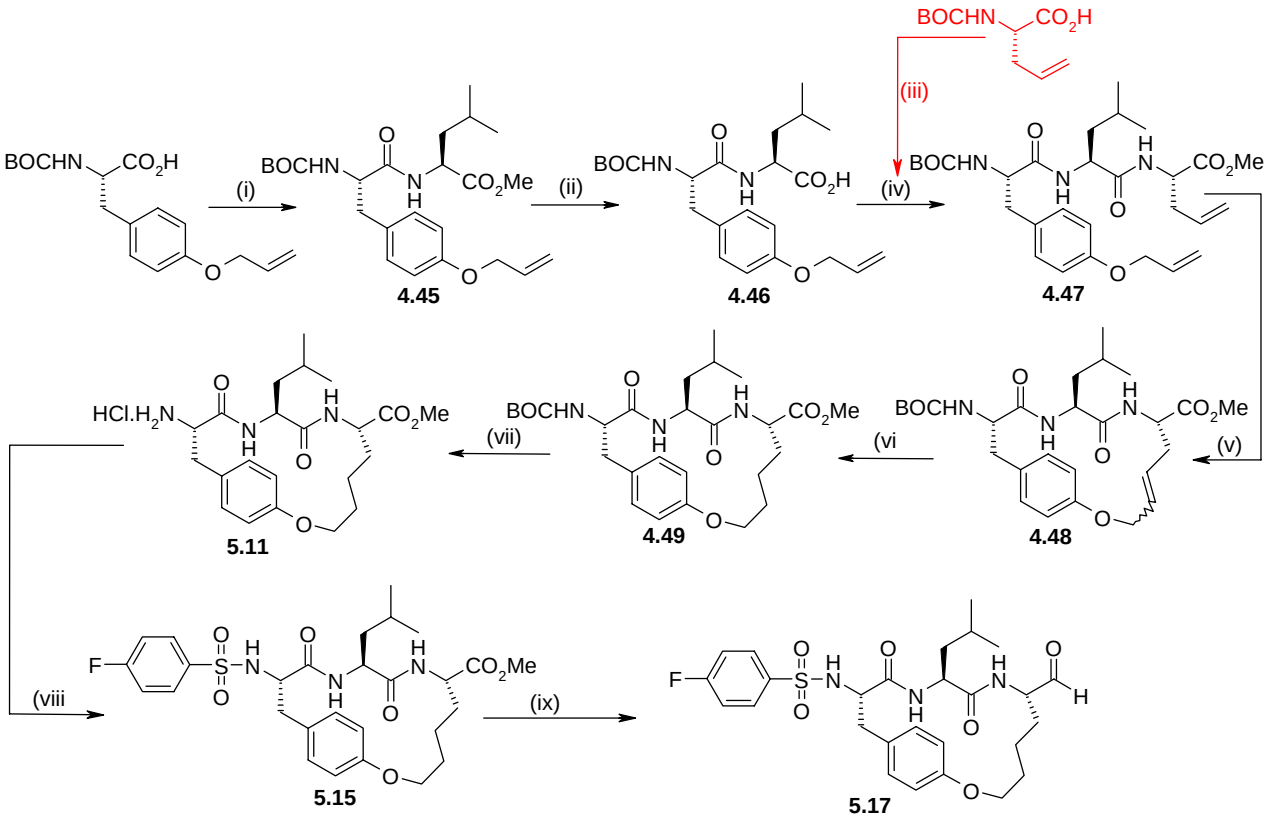
(1) Anterior suture lines **(2)** Anterior & posterior suture lines **(3)** 0-33% cortical nuclear involvement **(4)** 33-66% cortical nuclear involvement **(5)** Greater than 66% cortical nuclear involvement **(6)** Total immature cataract **(7)** Total mature cataract

Figure 6.2: One to seven cataract scoring system

6.2: Synthetic optimisation of 5.17 (Cat-812)

In order to evaluate the efficacy of our calpain inhibitors as anti-cataract agents in the *in-vivo* sheep model two potential administration routes were identified, intravitreal injection and topical administration (see **Section 5.3**). Compound **5.17** was identified as an intravitreal candidate. This had a cLogP of -0.37 and an IC_{50} value of 45 nM against calpain II.

Multi-gram quantities of **5.17** were required for an *in-vivo* sheep trial. However, as shown in **Scheme 6.1**, the original synthetic route to **5.17** had an overall yield of 0.37%. Three synthetic steps contributed to the low yield. RCM afforded only a moderate yield of ring closed product (29%), formation of the sulfonamide was extremely low yielding (7%) and DIBAL reduction of the ester was poor (25%). As the two lowest yielding transformations were the final two steps of the synthesis this initial synthetic route was not suitable for the preparation of multi-gram quantities of **5.17**.

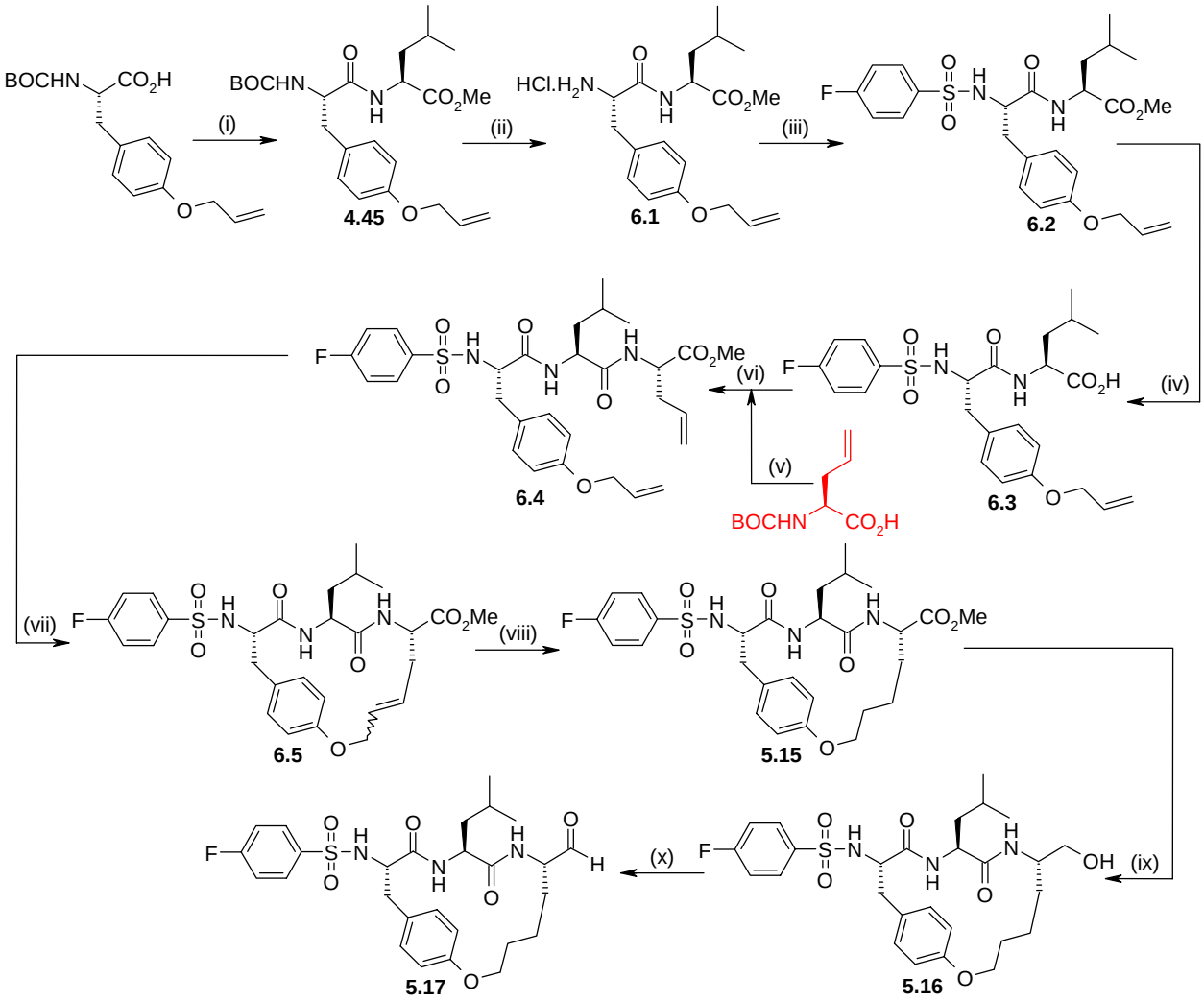


Scheme 6.1. *Reagents and Conditions:* **(i)** HATU, DIPEA, Leu-OMe, DMF, (80%); **(ii)** NaOH, THF, H_2O, MeOH, (97%); **(iii)** $SOCl_2$, MeOH, (100%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (97%); **(v)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (29%); **(vi)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (98%); **(vii)** 4M HCl, 1,4-dioxane, (100%); **(viii)** 4-fluorobenzene sulfonyl chloride, DIPEA, DMF, (7%); **(ix)** DIBAL, DCM, (25%); **Overall yield – 0.37%**

As shown in **Scheme 6.2** the overall yield of the synthetic route to **5.17** was improved from 0.37% to 16%. This was achieved by optimising two synthetic transformations and changing the order of the synthetic steps.

The yield of the sulfonamide formation reaction was improved by moving this step to earlier in the synthesis (from 7% to 65%). The yield of the RCM reaction was increased from 29% to 65% by adding 10 mol% of Lewis acid chloro-dicyclohexyl borane to the microwave irradiated reaction media (described in **Section 4.19**). The aldehyde was obtained by a

reduction, oxidisation protocol. Although this required an extra step it increased the yield of the ester to aldehyde transformation from 25% to 59%.

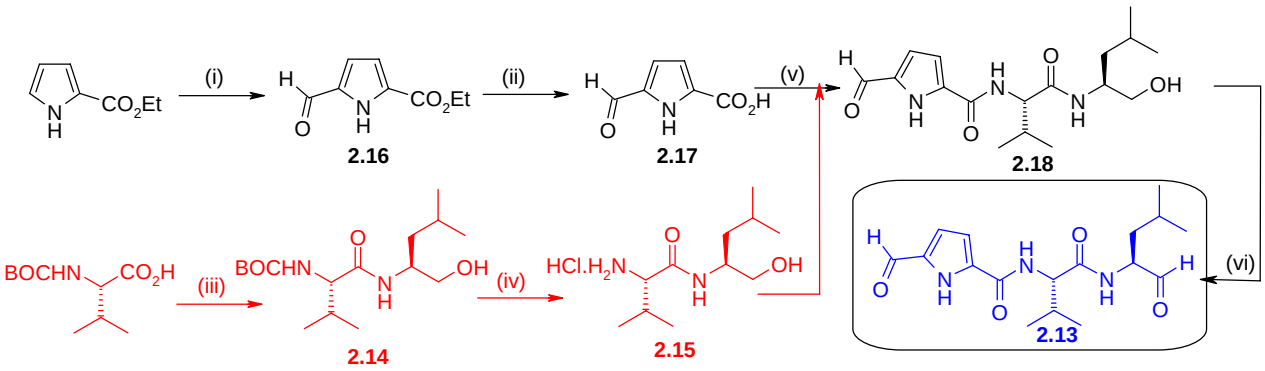


Scheme 6.2. *Reagents and Conditions:* **(i)** HATU, DIPEA, Leu-OMe, DMF, (80%); **(ii)** 4M HCl, 1,4-dioxane, (100%); **(iii)** 4-fluorobenzene sulfonyl chloride, DIPEA, DCM, (65%); **(iv)** NaOH, THF, H_2O, MeOH, (95%); **(v)** $SOCl_2$, MeOH, (100%); **(vi)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (89%); **(vii)** 3 x 10 mol% **3.9**, 10 mol% chloro-dicyclohexyl borane, 1,1,2-TCE, microwave, (65%); **(viii)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (93%); **(ix)** $LiAlH_4$, THF, (86%) **(x)** SO_3.Pyr, DIPEA, DMSO, DCM (69%); **Overall yield – 16%**

6.3: Synthesis of 2.13 (CAT-0059) for *in-vivo* evaluation

As described in **Section 5.2** the optimal route of administration for a human cataract drug is topical drug delivery. Compound **2.13** was identified as our best acyclic topical candidate with an IC_{50} of 30 nM and the required balance of physicochemical properties. It had a cLogP

of 0.90 and moderate aqueous solubility of 0.1 mg/ml. As shown in **Scheme 6.3** no optimisation of the synthetic route was required as it was already designed as a divergent synthesis (**Chapter 2**).



Scheme 6.3. *Reagents and Conditions:* **(i)** $POCl_3$, DMF, DCE, (52%); **(ii)** KOH, H_2O, (96%); **(iii)** HATU, DIPEA, (L)-leucinol, DMF, (68%); **(iv)** 4M HCl in 1,4-dioxane, (100%); **(v)** EDC, HOBt.H_2O, DIPEA, **2.15,** DCM (39%); or HATU, DIPEA, **2.15**, DMF (27%); **(vi)** SO_3.Pyr, DIPEA, DMSO, DCM, (66%)

6.4: Results of intravitreal trial of 2.13 (Cat 0059)

Prior to commencing a full scale *in-vivo* sheep trial it was deemed prudent to ensure that compounds administered by intravitreal injection diffused to the site of action (the lens). To investigate this our collaborators at Lincoln University administered an intravitreal injection of **2.13** to five sheep. These were sacrificed at 1, 6, 24, 72 and 168 hours. HPLC and LCMS analysis of the vitreous humor, aqueous humor and lens was performed to detect whether or not **2.13** was present. Results of this analysis are shown in **Table 6.1**

Time (h) / Tissue	1	6	24	72	168
2.13 detected in vitreous humor	Yes	Yes	No	No	No
2.13 detected in aqueous humor	No	No	No	No	No
2.13 detected in the lens	No	No	No	No	No

Table 6.1: Detection of **2.13** after intravirtreal injection

The results in **Table 6.1** clearly demonstrate that intravitreal injection is not a suitable method of drug delivery for compound **2.13**. The drug was only detected in the vitreous humour during the course of the experiment **2.13** and it was not detected, at any time, either in the

aqueous humor or at the site of action (the lens). Furthermore **2.13** was cleared entirely from the vitreous humor between six and twenty four hours. For these reasons intravitreal injection was rejected as a route of administration for our calpain inhibitors as anti-cataract agents.

6.5: *In-vivo* sheep trial results of 2.13 (CAT-0059)

Two separate *in-vivo* trials were conducted by the Lincoln University cataract group using **2.13**. In October 2004 a ten week trial using an eye drop formulation of **2.13** comprised of the following composition (see **appendix 2**) was conducted;

- 0.1% (w/w) compound **2.13**
- 14% EtOH
- 0.9% sodium chloride
- 0.3% hydroxypropyl methyl cellulose
- 0.05% disodium EDTA
- 0.01% benzalkonium chloride
- 84.65% MilliQ purified water

The calpain inhibitor was applied to the left eye of cataract lambs, leaving the right eye as an untreated control. The progression of cataracts in both eyes was determined by a veterinary ophthalmologist using a slit-lamp microscope. Each eye was assigned a cataract score (**see Section 6.2**). The cataract scores were taken two weeks before the start of the evaluation (t= -14) and three times during the eleven weeks of treatment (t = 10, 37, 67 days). The mean score after each examination is shown in **Figure 6.4**.

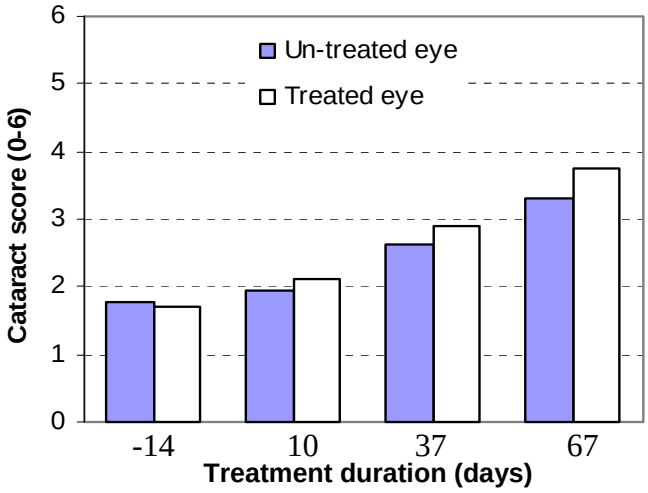


Figure 6.4. A plot of mean cataract scores for untreated and treated eyes

The cataract scores of the treated and untreated eyes were the same over the first thirty seven days of the treatment period. At the end of the treatment period, the eyes treated showed a higher cataract score than the untreated eye ($P < 0.05$) thus it was concluded that **2.13** did not retard cataract development under these conditions.

The reason for this failure was thought to be due to insufficient diffusion of **2.13** through the cornea into the anterior of the eye. The use of ethanol in the eye drop formulation resulted in an eye drop which had extremely low surface tension. This resulted in the majority of the eye drop immediately being washed off the surface of the eye.

As such another trial was undertaken in October 2005 using **2.13** formulated into an ointment. It was anticipated that an ointment would remain on the surface of the eye for a longer period of time compared to an eye drop. Importantly, the ointment preparation enabled a ten fold higher concentration of **2.13** to be used relative to the eye drop. It was hypothesised that both the increased concentration of **2.13** and the fact that the ointment was much more viscous would result in an increased concentration of **2.13** residing on the eye for a longer period of time. In turn it was expected that this would allow more time for diffusion across the cornea

and thus this would result in a very significant increase in the concentration of **2.13** in the anterior of the eye. The formulation was prepared (see **appendix 2**) using the following composition (w/w):

- 1% compound **2.13**
- 25% cetyl stearyl alcohol
- 35% lanolin
- 39% paraffin oil.

The trial began on 16/11/05 and continued until 26/2/06. Twenty-four two to three month old lambs were selected for the trial based on their cataract score on 28/10/06. Their cataract scores ranged from one to four with means of 1.92 for the left eye and 1.96 for the right. The left eye of each sheep was treated twice daily with twenty five milligrams of ointment containing 0.25 milligrams of **2.13**.

During the trial the cataracts progressed rapidly and by 22/2/06 over half had mature cataracts (**Table 6.2**). This data is also shown graphically in **Figure 6.5**.

Date	Number	Left Eye	Right Eye	Left Std Dev	Right Std Dev
28-Oct	24	1.92	1.96	0.58	0.69
29-Nov	24	3.17	3.71	1.58	1.85
Dec-07	24	3.42	3.75	1.61	1.73
Dec-21	22*	4.41	4.45	1.82	2.09
Jan-09	24	4.79	4.79	1.93	2.00
Jan-20	23	5.43	5.13	1.93	1.91
Feb-22	22	5.95	5.86	1.56	1.67

* two animals could not be scored because of eye infection

Table 6.2: Results of **2.13** ointment *in-vivo* cataract sheep trial.

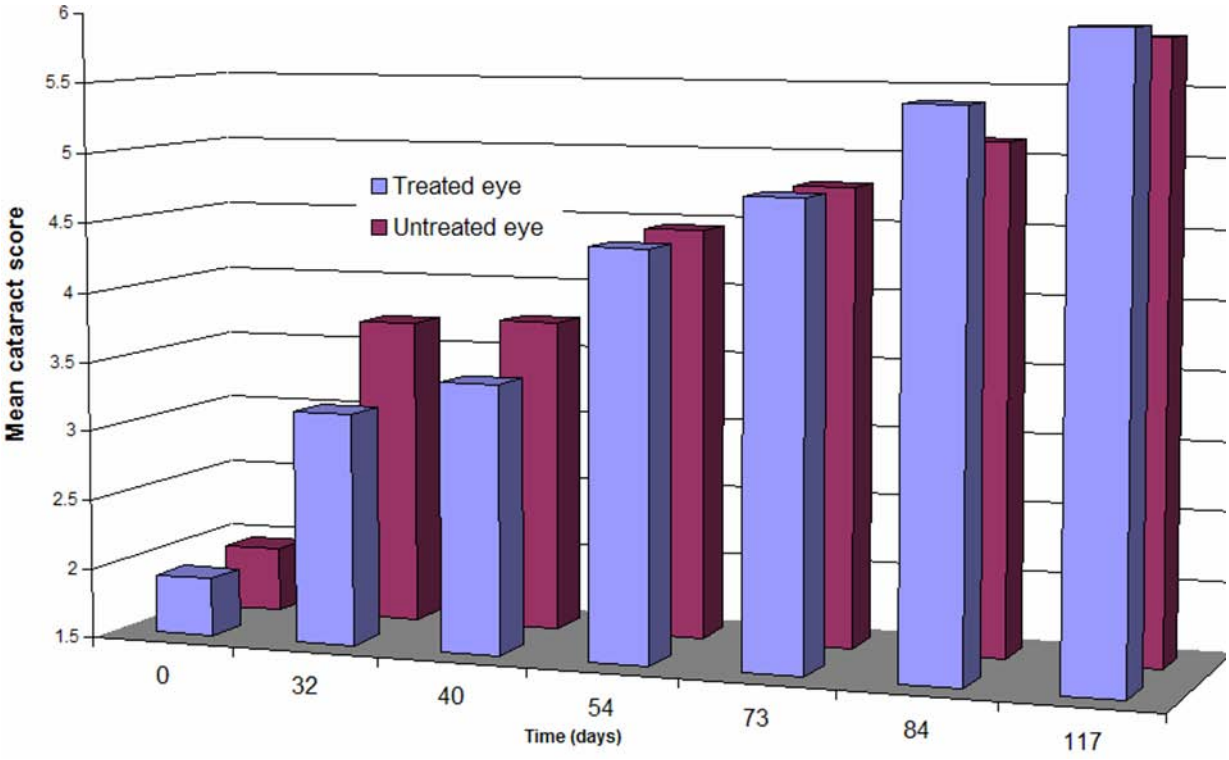


Figure 6.5: Results of **2.13** ointment *in-vivo* cataract sheep trial.

After one month the treated left eyes showed an average cataract score of 3.17 and the control right eyes showed an average cataract score of 3.71. This was significant in a paired t-test at $p < 0.05$ indicating successful retardation of cataract development.

This result is very promising in the context of developing a human cataract drug. Human cataracts typically develop much slower than those within the sheep trial. It is postulated that the statistically significant delay of cataract development for one month in the *in-vivo* sheep cataract model would translate into a much longer period of time in humans.

In the developed world cataract is readily treated by surgery. However, there are frequently long waiting lists for cataract operations and during this time the individual usually receives no treatment and as such cataract development progresses unabated. The results from this trial suggest that development of a compound, such as **2.13**, which might slow down the rate

of cataract development for a number of months or even years would greatly improve the quality of life for individuals awaiting cataract surgery.

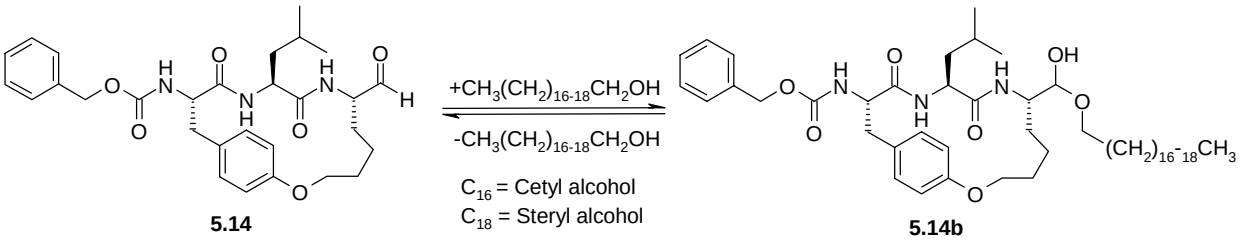
Furthermore, from this *in-vivo* trial it can also be concluded that correct formulation of the drug is imperative for successful treatment. Use of **2.13** as an anti-cataract agent in the form of an eye drop was totally unsuccessful whereas use of **2.13** as an ointment resulted in a statistically significant reduction in the rate of cataract formation. This ointment formulation of **2.13** shows promise as a potential treatment for cataract.

6.6: Synthetic optimisation of 5.14 (CAT-811)

As described in **Section 5.2** the optimal route of administration for our inhibitors is topical drug delivery. The macrocyclic inhibitors were designed using LogP calculations to optimise cornea penetration. As such a macrocyclic inhibitor was required for *in-vivo* evaluation which was both potent and with a LogP in the target range of one to three. Compound **5.14** was chosen with a cLogP of 1.59 and an IC_{50} value of 27 nM against calpain II. This was formulated into an ointment (see **appendix 2**), suitable for intraocular application, with a optimised formulation comprised of (w/w):

- 1% compound **5.14**
- 25% cetyl stearyl alcohol
- 35% lanolin
- 39% paraffin oil

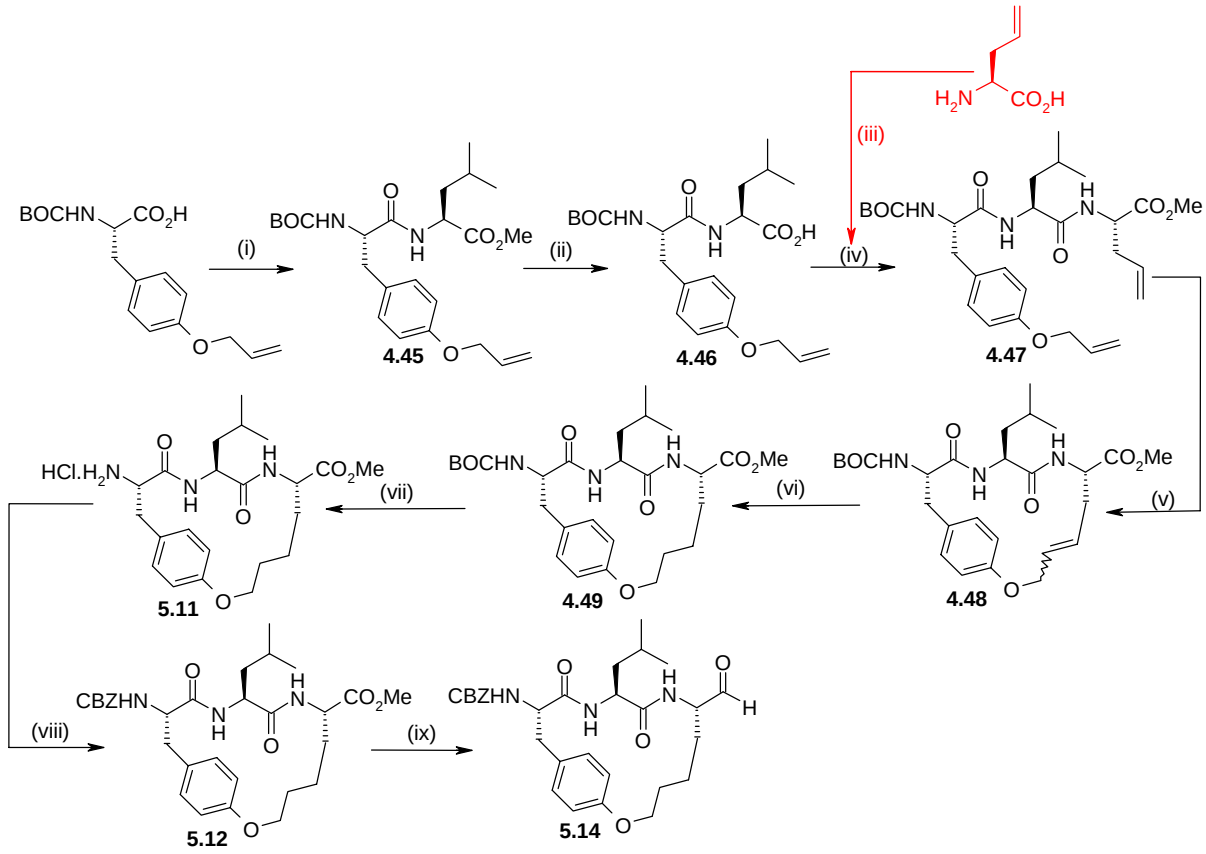
The experimental procedure for the preparation of ointment of **5.14** required the aldehyde to first be dissolved in cetyl stearyl alcohol. Mass spectrometry analysis of this initial solution indicated that **5.14** was in equilibrium with its hemi-acetal **5.14b** (**Scheme 6.4**).



Scheme 6.4: Hemi-acetal equilibrium of **5.14**

This observation is critical to the interpretation of the *in-vivo* trial results. Formation of hemi-acetal **5.14b** results in an increase in the clogP of **5.14** (from 1.59 to 5.48 and 5.61 respectively). As discussed in **Section 5.3** logP is directly related to cornea permeability. From **Figure 5.5** it is seen that the increase in logP, upon formation of the hemi-acetal, is predicted to result in a three fold increase in the percentage of compound which reaches the anterior side of the eye. This is a significant advantage for cataract treatment and as such this represents a pseudo pro-drug approach

The use of **5.14** in this formulation required the synthesis of multi-gram quantities of **5.14**. As shown in **Scheme 6.5** the initial synthetic route of **5.14** had an overall yield of 2.0%.

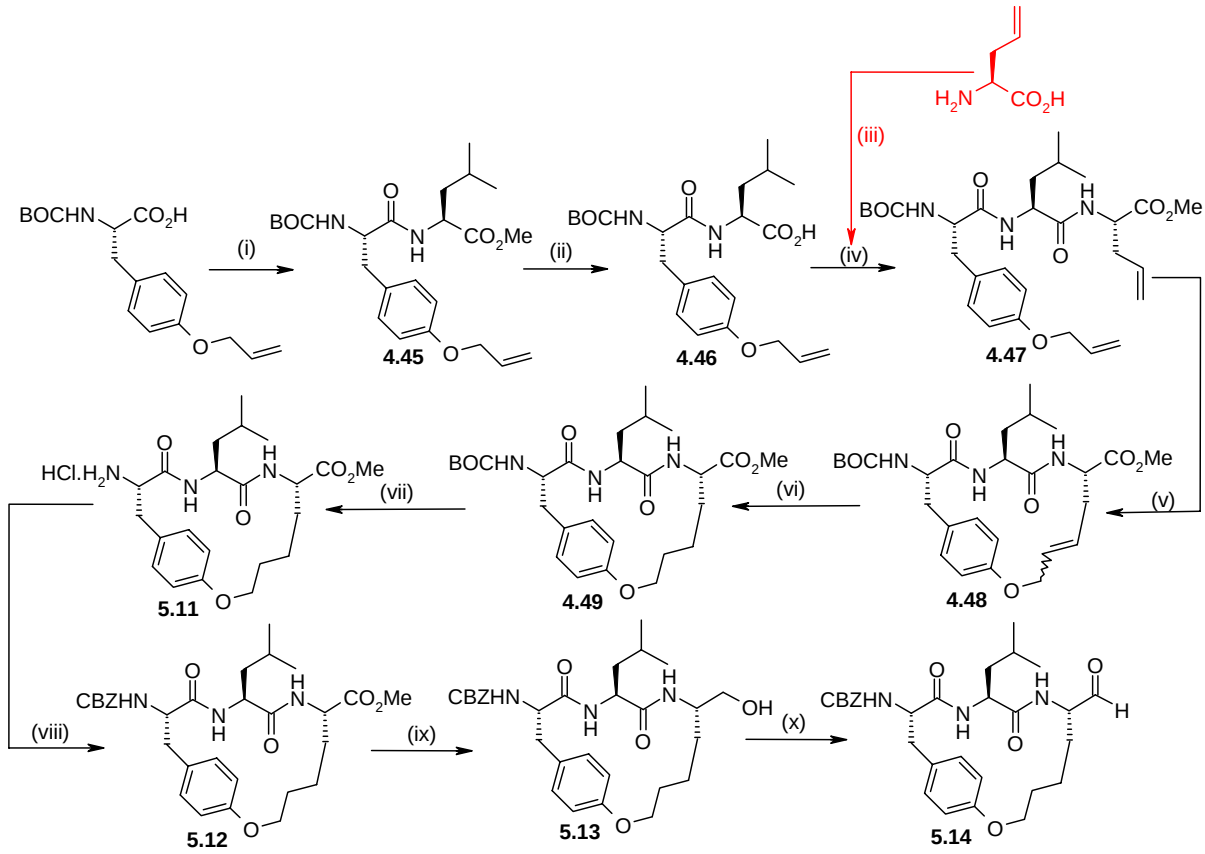


Scheme 6.5. *Reagents and Conditions:* **(i)** HATU, DIPEA, Leu-OMe, DMF, (80%); **(ii)** NaOH, THF, H_2O, MeOH, (97%); **(iii)** $SOCl_2$, MeOH, (100%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (97%); **(v)** 3 x 10 mol% **3.9**, 1,1,2-TCE, microwave, (29%); **(vi)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (98%); **(vii)** 4M HCl, 1,4-dioxane, (100%); **(viii)** benzyl chloroformate, DIPEA, DMF, (43%); **(ix)** DIBAL, DCM, (22%); **Overall yield – 2.0%**

The low overall yield is the result of a nine step, almost linear, synthetic route with three low to moderate yielding steps (RCM, *N*-CBZ formation and reduction of the ester to the aldehyde). The order of the synthetic steps could not be changed as hydrogenation of the double bond in **4.48** was required. Reaction conditions which would also cleave a *N*-CBZ functionality. As such the synthetic optimisation strategy was to improve the yield of the individual steps.

As shown in **Scheme 6.6** the RCM reaction yield was improved from 29% to 91% by addition of 10 mol% of chloro-dicyclohexyl borane to the microwave irradiated RCM reaction media.

The ester reduction to aldehyde was also improved from 22% (using DIBAL) to 35% using a lithium aluminium hydride reduction, sulfur trioxide oxidation sequence. Combined the improvement of yield in these two steps increased the overall yield five fold (10% compared to 2%). This sequence then allowed preparation of two grams of **5.14**.



Scheme 6.6. *Reagents and Conditions:* **(i)** HATU, DIPEA, Leu-OMe, DMF, (80%); **(ii)** NaOH, THF, H_2O, MeOH, (97%); **(iii)** $SOCl_2$, MeOH, (100%); **(iv)** HATU, DIPEA, (s)-allyl-Gly-OMe, DMF, (97%); **(v)** 3 x 10 mol% **3.9**, 10 mol% chloro-dicyclohexyl borane, 1,1,2-TCE, microwave, (91%); **(vi)** H_2, 20 mol% Pd/C, MeOH, EtOAc, (98%); **(vii)** 4M HCl, 1,4-dioxane, (100%); **(viii)** benzyl chloroformate, DIPEA, DMF, (43%); **(ix)** $LiAlH_4$, THF, (83%); **(x)** SO_3.Pyr, DIPEA, DMSO, DCM, (42%); **Overall yield – 10%**

6.7: *In-vivo* sheep trial results of 5.14 (CAT-811)

The protocol used for this trial was the same as for the **2.13** ointment trial. The trial began on 16/12/05 and continued to 28/3/06. Twenty-four three to four month old lambs were selected for the trial based on their cataract score on 7/12/05. Their initial scores ranged from 1 to 2

with means of 1.50 for the left eye and 1.54 for the right. Two lambs died during the trial and a further two were sacrificed in an attempt to measure levels of **5.14** in the eyes. The left eye of each sheep was treated twice daily with twenty five milligrams of ointment containing 0.25 milligrams of **5.14**. (**Table 6.3** and **Figure 6.6**).

Date	Number	Left Eye	Right Eye	Left Std Dev	Right Std Dev
Oct-28	24	0.71	0.61	0.62	0.58
Dec-07	24	1.5	1.54	0.51	0.59
Dec-21	23	1.57	1.55	0.66	0.67
Jan-09	23	2.17	1.96	1.15	0.82
Jan-20	23	2.26	2.26	1.14	1.1
Feb-22	22	2.68	2.5	0.95	0.67
Mar-20	20	3.2	3.05	1.44	1

Table 6.3: Results of **5.14** ointment *in-vivo* cataract sheep trial.

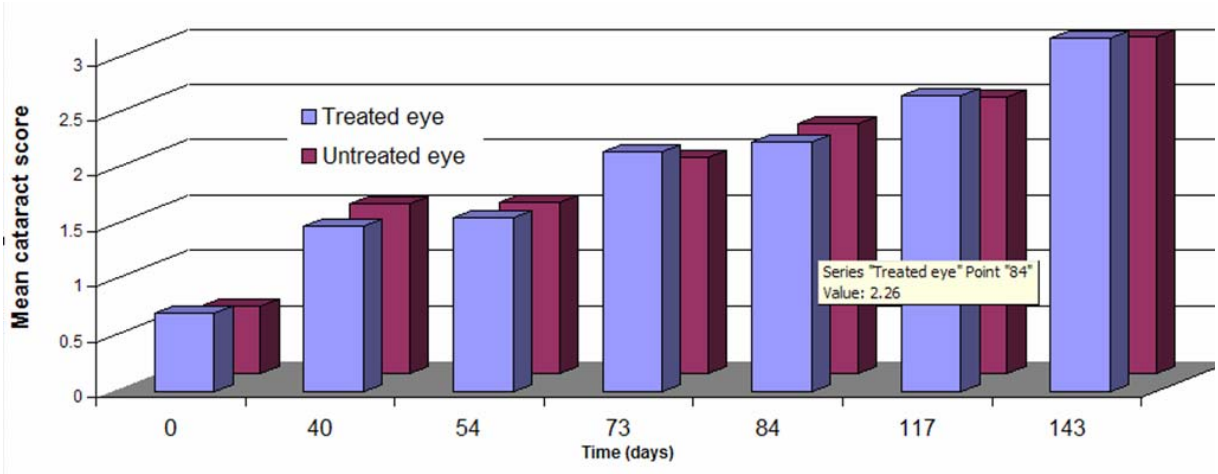


Figure 6.3: Results of **5.14** ointment *in-vivo* cataract sheep trial.

A key finding from the trial is that progression of cataracts in both eyes was very slow and by the last measurement, after 143 days, none of the sheep had progressed to mature cataracts and only two eyes had total immature cataracts. These are unprecedented results as no chemical compound has ever been used to so dramatically reduce the rate of cataract development.

The fact that there was no significant difference between the eyes at any point in the trial can be explained by cross over of drug between the eyes. This is a recognised but poorly understood phenomenon. To support this hypothesis it should be noted that our collaborators have previously demonstrated[1] that between twenty and forty per cent of **2.13** was detected in the untreated eye of the cataract sheep flock from a previous *in-vivo* efficacy trial. Moreover, there is literature precedence to explain such an observation. The application of glaucoma drug timolol maleate to albino rabbits resulted in lowering of intraocular pressure in the contralateral eye by 20-40% relative to the treated eye.[2] Further work is required to establish the mechanism of, and quantify, cross over of **5.14** between the eyes

In order to understand this data the rate of progression of cataracts in this trial was evaluated The progression of cataract was compared with data from other trials. Four comparable data sets were identified;

- control animals from a trial undertaken with SJA 6017 in 2000
- the untreated right eye from the SJA 6017 trial in 2000
- the untreated right eye from two trials in 2004.

The weekly rate of cataract score progression for all five groups of animals was calculated (**Table 6.4).** This data is also represented graphically in **Figure 6.7**.

Group	Number of animals	Rate of cataract score progression (per week)
Untreated eye (**5.14** trial)	20	0.1064
Untreated eye (Control animals)	9	0.1890
Untreated eye (SJA 2000 trial)	14	0.2093
Untreated eye (first 2004 trial)	9	0.1953
Right eye (second 2004 trial)	12	0.2037

Table 6.4: Daily rate of cataract score progression

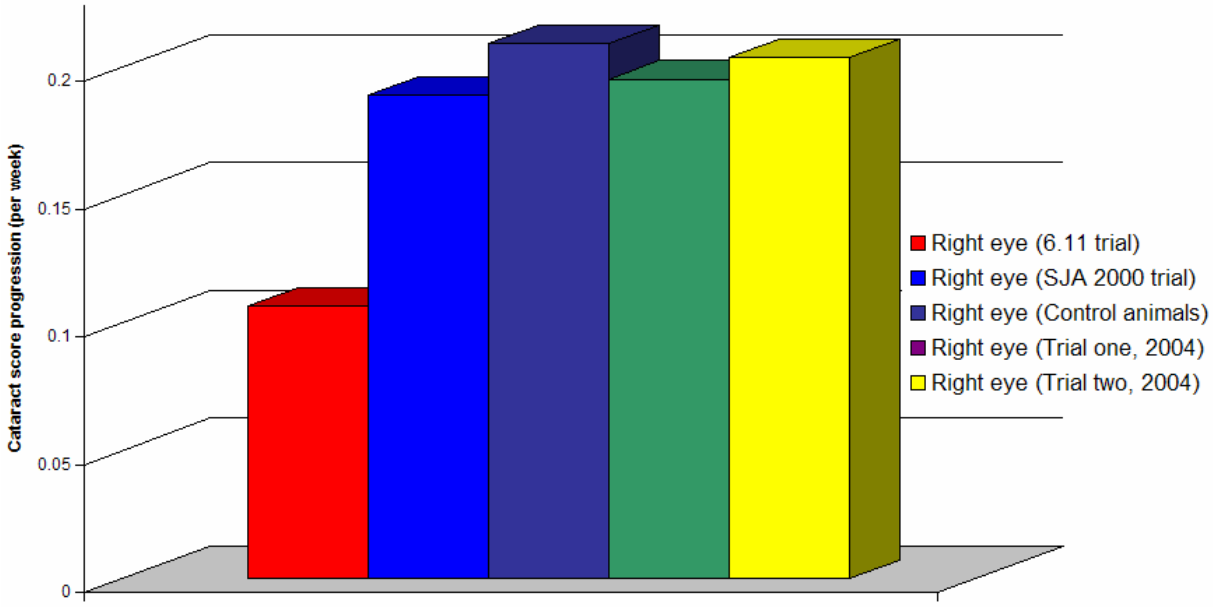


Figure 6.7: Weekly rate of cataract score progression

Sheep treated with **5.14** had a weekly cataract score progression of 0.1064 and those not treated with **5.14** had weekly cataract progression scores of between 0.1890 and 0.2093. From the data it is clear that the rate of cataract formation in animals treated with **5.14** was markedly decreased.

The data obtained from the *in-vivo* trial indicates that **5.14** is efficacious. Administration of **5.14** slowed the rate of cataract formation between forty four and forty nine per cent relative to control animals. The next *in-vivo* trial to commence in September 2006 will re-investigate the use of **5.14** as an anti-cataract agent. This trial will be constructed as a double blind placebo controlled drug efficacy study. Two groups of lambs will be treated with drug or placebo respectively. Neither our collaborators or the veterinary ophthalmologist will be aware of which group are being administered the drug and which are receiving placebo. Data from this work will be used to confirm whether or not **5.14** has the potential to be developed as an anti-cataract drug.

6.8: Conclusions and further work

The synthetic routes to three calpain inhibitors (**2.13**, **5.14** and **5.17**) were optimised and used to synthesise multi-gram quantities of each. Two of these (**2.13** and **5.14**) were evaluated as anti-cataract agents *in-vivo* using the cataract sheep model. Both of these β-strand mimics were demonstrated to retard cataract development. Macrocycle **5.14** was found to be the most effective, decreasing the rate of cataract development between forty four and forty nine per cent relative to control.

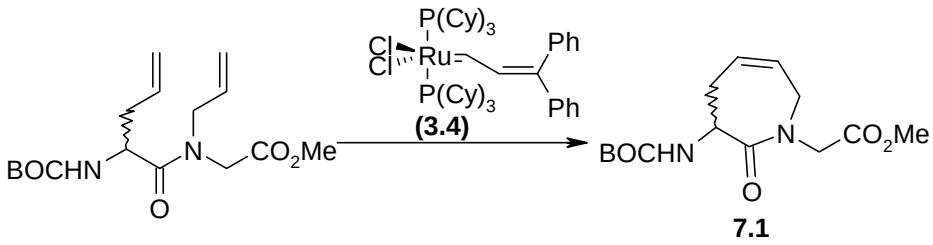
Intravitreal injection was determined to be an inappropriate route of administration for our calpain inhibitors. This was concluded from a study where compound **2.13** was injected into five sheep and *ex-vivo* LCMS and HPLC analysis showed that **2.13** did not to diffuse to the site of action (the lens).

References for Chapter 6

1. Robertson Lucinda, J. G.; Morton James, D.; Yamaguchi, M.; Bickerstaffe, R.; Shearer Thomas, R.; Azuma, M., Calpain may contribute to hereditary cataract formation in sheep. *Investigative ophthalmology & visual science* **2005,** 46, (12), 4634-40.
2. Aggarwal, D.; Kaur, I. P., Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system. *International Journal of Pharmaceutics* **2005,** 290, (1-2), 155-159.

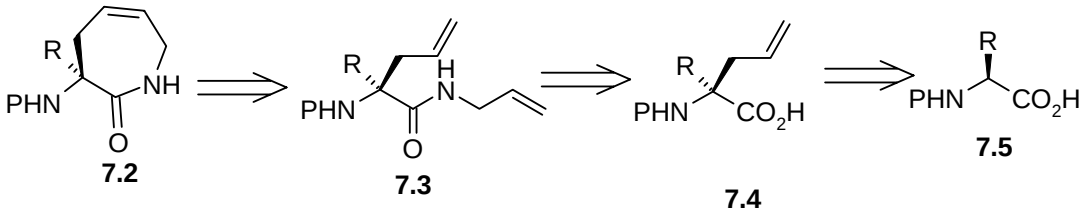
7.1: Synthesis of α-α conformationally constrained peptidomimetic lactams

A key concept in peptidomimetic research (see **Section 3.1)** is that conformation defines biological activity. Lactam rings have found extensive use in the medicinal and pharmaceutical industry as a means of conformational constraint.[1-3] Given the success of our RCM methodology we chose to investigate the synthesis of peptide-based lactams using RCM. A 1996 paper by Grubbs *et al.*[4] demonstrates this approach with the synthesis of racemic glycine based lactams such as **7.1** (**Scheme 7.1**).



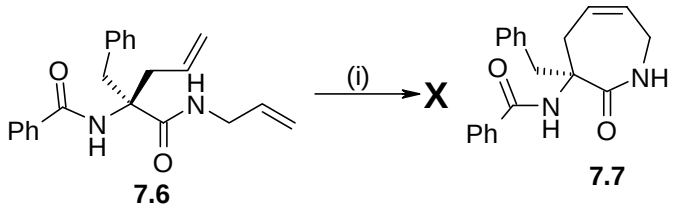
Scheme 7.1. *Reagents and Conditions:* **(i)** 5 mol% **3.4,** $CHCl_3$, 52%

The creation and implementation of a synthetic strategy which would provide a stereoselective route to novel α-α disubstituted seven-membered lactams of general type **7.2** was sought in our laboratory. As shown in **Scheme 7.2** retrosynthetic analysis was performed to aid synthetic route design.



Scheme 7.2. Retrosynthesis of lactam **7.2**

Work on this type of compound was commenced by Dr J. Gardiner. As shown in **Scheme 7.3**, diene **7.6** was successfully prepared but RCM failed.



Scheme 7.3. *Reagents and Conditions:* **(i)** 10 mol% **3.5**, range of conditions (DCM, rt; or benzene, rt; or benzene, reflux), (all 0%)

The reason for the failure of RCM was thought to be due to diene **7.6** adopting an extended conformation, with the terminal olefin *trans* to the C1-N1 amide bond **(Figure 7.1).** As such it was hypothesised that the olefins were too distant to undergo RCM

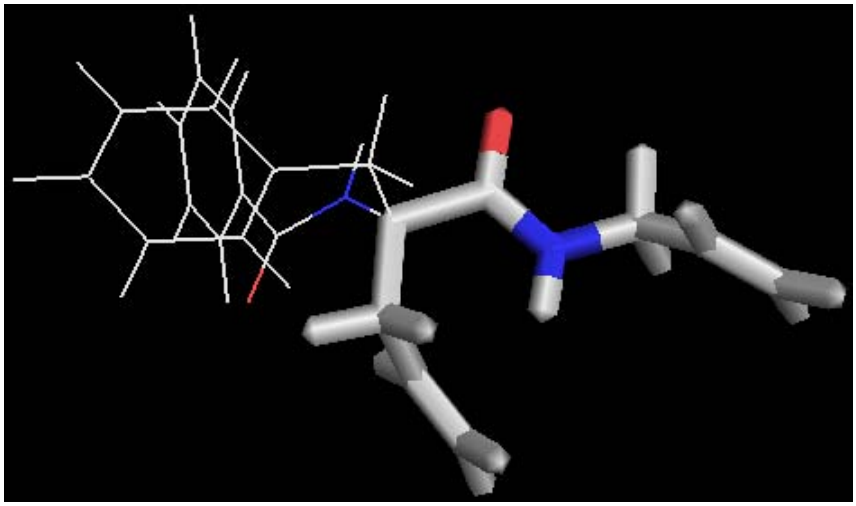
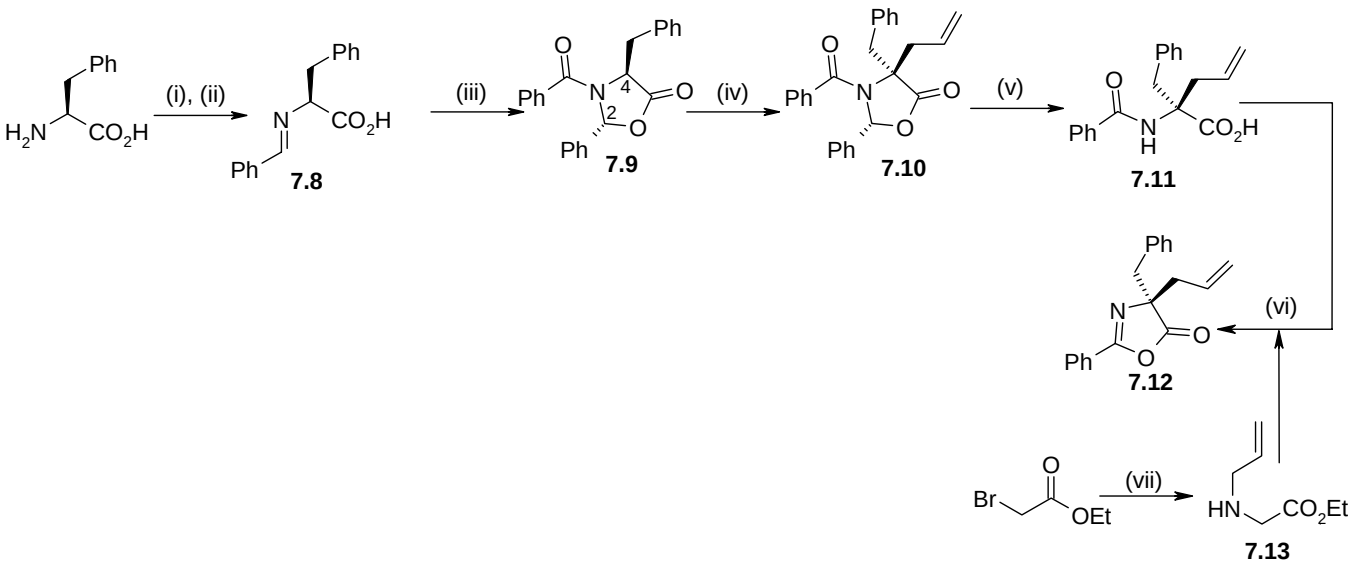


Figure 7.1: *Trans* relationship of a diene across the C1-N1 amide bond

Replacement of the amide proton with 2,4-dimethoxybenzene has been used successfully[5] in the Abell group to favour a *cis* relationship of olefins across a C1-N1 amide to promote RCM. As such it was postulated that substitution of the allyl amide nitrogen in **7.6** would favour a *cis* relationship of olefins across the C1-N1 bond and thus this would allow productive RCM.

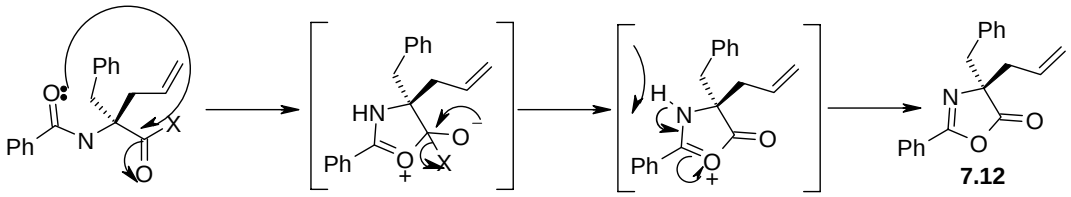
The first attempted synthesis of a *N*-substituted diene was performed using a synthetic strategy that was well established in the Abell group (**Scheme 7.4**).[6-10] The sodium salt of (L)-phenylalanine was condensed with benzaldehyde with azetropic removal of water to give

Schiff base **7.8**. Acylation of this with benzoyl chloride gave pure *trans*-5-oxazolidinone **7.9**. The assignment of *trans* configuration to **7.9** was based on literature NMR data.[10] Stereoselective allylation of **7.9** with allyl bromide, followed by hydrolysis under basic conditions gave carboxylic acid **7.11.** Attempted coupling of *N*-allyl amine **7.13** to carboxylic acid **7.11**, under a wide variety of conditions, gave side product oxazol-5-one **7.12** exclusively.



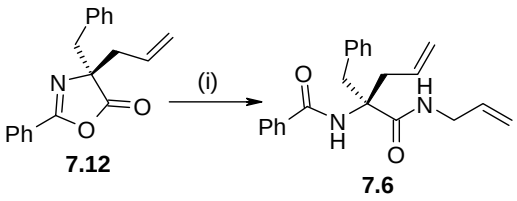
Scheme 7.4. *Reagents and Conditions:* **(i)** $NaOH_{(aq)}$; **(ii)** PhCHO, DCM, reflux (95%, (over 2 steps)); **(iii)** PhCOCl, DCM, 0°C (35%); **(iv)** LiHMDS, allyl bromide, THF, -78°C (81%); **(v)** NaOH, MeOH, H_2O (100%); **(vi)** a) Cyanuric fluoride, Pyr, DCM; b) **7.13**, DCM; or HATU, DIPEA, **7.13**, DMF; or HATU, DIPEA, **7.13**, THF; or EDC, HOAT, DIPEA, **7.13**, DMF; or HATU, HOAT, DIPEA, **7.13**, DMF or PyAOP, DIPEA, **7.13**, DMF; or PyBroP, DIPEA, **7.13**, DCM (all 0% for desired coupling, 0-100% of **7.12**); **(vii)** Allylamine, THF, (73%)

Scheme **7.5** shows a possible mechanism for the intramolecular cyclisation of **7.11** to give oxazol-5-one **7.12**. Cyclisation occurs by carbonyl nucleophilic attack at the electrophilic centre (X is either an HOAT ester or F). Literature supports this mechanism.[11]



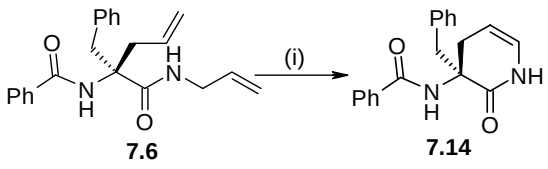
Scheme **7.5:** Mechanism for the intramolecular cyclisation

With oxazol-5-one **7.12** in hand, and as an alternative synthesis to prepare diene **7.6**, the anion of allyl amine was used successfully to ring open oxazol-5-one **7.12 (Scheme 7.6).** Ring opening of oxazol-5-one **7.12** was also attempted using the anion of *N*-allyl amine **7.13.** However, only starting material was recovered in quantitative yield. The reason for this is postulated to be that the amine is simply too sterically hindered to react.



Scheme 7.6. *Reagents and Conditions:* **(i)** nBuLi, THF, -78^{o}C, allyl amine, (96%) or *N*-allyl-Gly-OEt (0%).

The failure of diene **7.6** to cyclise using Grubbs first generation catalyst was reported by Dr J Gardiner**.** This thesis now reports the cyclisation of diene **7.6** using Grubbs second generation catalyst in refluxing benzene (**Scheme 7.7**). This unexpectedly resulted in a ring contraction reaction and formation of six membered lactam ring **7.14**.



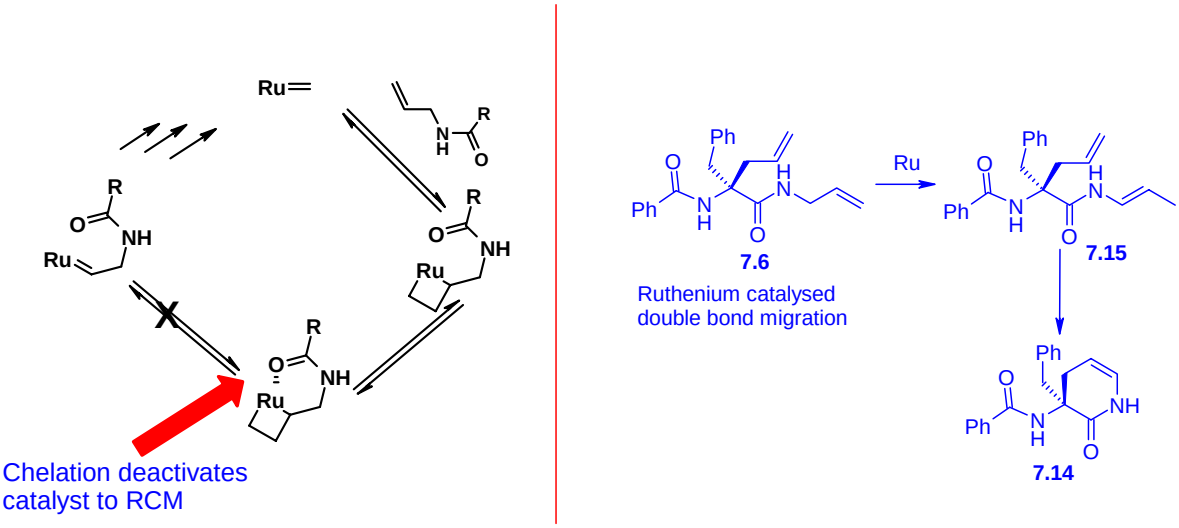
Scheme 7.7. *Reagents and Conditions:***(i)**10 mol% **3.9,** benzene, reflux, o.n (32%)

Data presented in **Section 4.16** suggest that failure of **7.6** to undergo RCM to form the seven membered ring is probably due to the formation of a stable catalyst de-activating six-membered chelate rather than the previous postulated steric reasons. Chelation can also be used to account for the formation of the ring contracted product.

Ring contraction is postulated to be due to a ruthenium catalysed double bond migration (**7.6** to **7.15**) followed by RCM (**7.15** to **7.14**). This is unusual as under normal circumstances RCM reactions are faster than the competing double bond migration reaction.

In this thesis it is proposed that dienes such as **7.6** form a six membered catalyst deactivating chelate with Grubbs second generation catalyst (**Scheme 7.8** and discussed in much more detail in **Section 4.16**). This chelate prevents productive RCM and as such the "slow" (as compared to usual RCM reactions) ruthenium catalysed double bond migration occurs exclusively. Double bond migrated diene **7.15** no longer forms a six-membered catalyst deactivating chelate and as such RCM to form the six membered lactam occurs quickly.

It would be worthwhile to repeat this reaction using the combined microwave irradiation/Lewis acid RCM reaction conditions **(see Section 4.16)** to determine if this would result in the formation of the desired seven membered lactam.

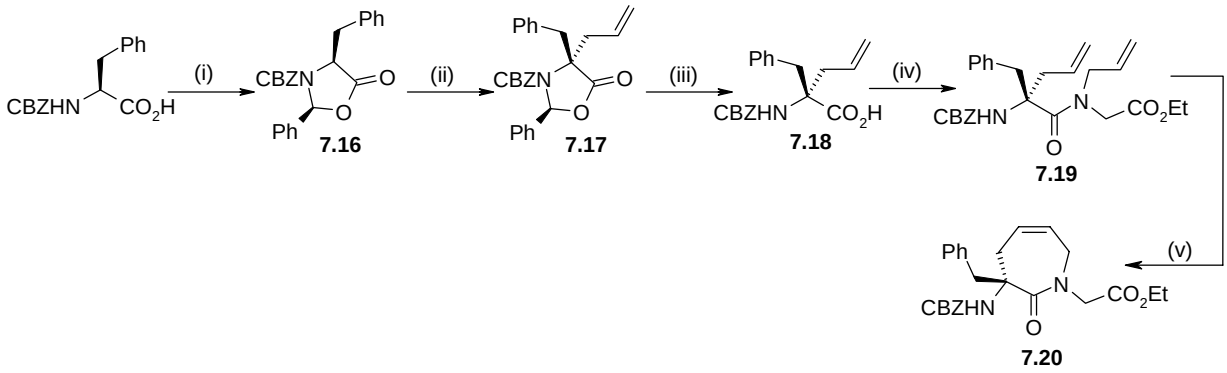


Scheme 7.8: Catalyst deactivation and subsequent RCM ring contraction reaction

This ring contraction reaction motivated further synthetic effort to prepare an *N*-amide substituted analogue of diene **7.6.** This was of interest as it was still thought that an *N*-amide substituent would facilitate productive RCM.

As formation of oxazolone **7.12** (**Scheme 7.4**) resulted from nucleophilic attack by the oxygen of the benzoyl group, an alternative nitrogen protecting group was required. As shown in **Scheme 7.9** benzyloxycarbonyl was employed as the nitrogen protecting group.

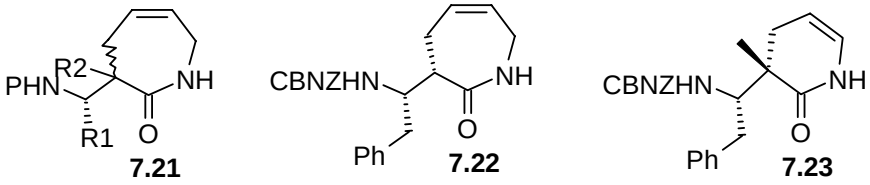
Diastereomerically pure *cis*-5-oxazolidinone **7.16** was prepared using a literature procedure[12] and assignment of *cis* to **7.16** was confirmed by comparison to literature NMR data.[12] Stereoselective allylation with allyl bromide afforded disubstituted oxazolidinone **7.17** and hydrolysis under basic conditions gave carboxylic acid **7.18.** Coupling of *N*-allyl amine **7.13** was achieved using PyAOP as the coupling reagent. However, ring closed product **7.20** was not obtained from RCM of diene **7.19**. Starting material was recovered in quantitative yield. This is postulated to be because diene **7.19** can still form the six membered catalyst deactivating chelate.



Scheme 7.9. *Reagents and Conditions:* **(i)** Benzaldehyde dimethylacetyl, $BF_3.OEt_2$, 4 Å sieves, ether, (31%); **(ii)** LiHMDS, allyl bromide, THF, -78^oC, (50%); **(iii)** NaOH, THF, H_2O, MeOH, (94%); **(iv)** PyAOP, DIPEA, **7.13**, DMF, (18%); **(v)** 10 mol% **3.9**, benzene, reflux, o.n (0%)

7.2: Synthesis of C-N conformationally constrained β-amino acid lactams

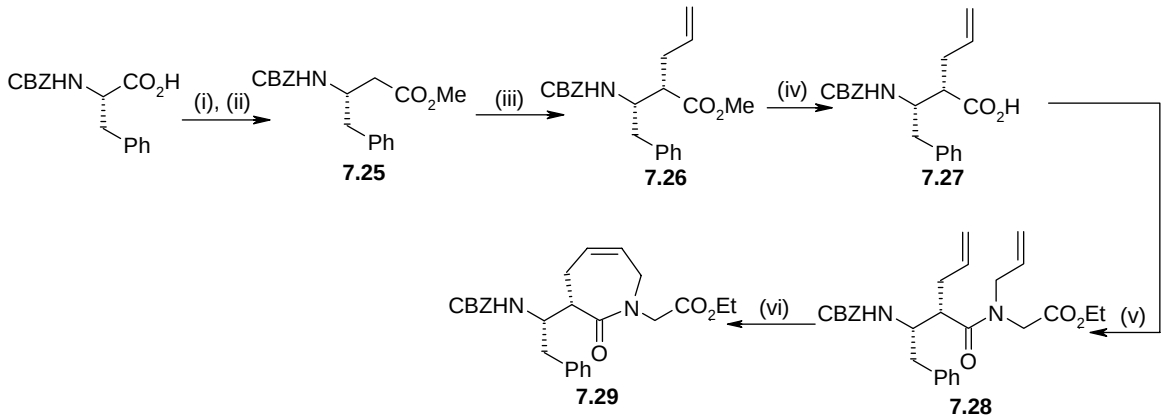
Recently β-amino acid containing peptides and peptidomimetics have generated considerable interest. In our laboratory the incorporation of β-amino acids into cyclic lactams has been extensively studied. We have developed methodology[13] to synthesise compounds of general type **7.21.** These can either be unsubstituted or substituted at the R^2 position. Compounds **7.22** and **7.23,** representative examples of this class, have been prepared by our group**.**



The objective of work contained in this thesis was to develop methodology to allow incorporation of **7.21** based motifs into peptides and peptidomimetics.

The synthesis of cyclic lactam **7.29,** shown in **Scheme 7.10**, provides an example of this methodology. The first step required conversion of *N*-CBZ protected phenylalanine into its corresponding β-amino acid methyl ester (**7.25**) under standard Arndt-Eistert homologation

conditions.[14] The enolate of methyl ester **7.25**, generated using LDA, was allylated with allyl bromide to give **7.26** stereospecifically. Hydrolysis of methyl ester **7.26,** and coupling of resultant carboxylic acid **7.27** with amine **7.13** gave diene **7.28.** RCM was achieved using Grubbs second generation catalyst in refluxing benzene to give lactam **7.29**.



Scheme 7.10. *Reagents and Conditions:* **(i)** $ClCO_2Et$, Et_3N, THF; **(ii)** CH_2N_2, AgOBn, Et_3N, MeOH, (48% over 2 steps); **(iii)** LDA, LiCl, allyl bromide, THF, (65%); **(iv)** NaOH, THF, MeOH, H_2O, (83%); **(v)** PyAOP, *N*-allyl-gly-OEt **7.13**, DIPEA, DMF, (29%); **(vi)** 10 mol% **3.9**, benzene, reflux, (22%)

7.3: Conclusions and future work

Methodology for the chiral synthesis of α-α disubstituted amino acid dienes such as **7.19** has been developed. However, RCM of diene **7.19** remained elusive. Further work in this area is needed to optimise the RCM reaction conditions for these types of substrates. The use of the combined microwave irradiation/Lewis acid reaction conditions developed in **Chapter 4** could potentially be used to ring close diene **7.19**.

A rare ring contraction RCM reaction was observed when diene **7.6** was subjected to Grubbs second generation catalyst. Instead of obtaining a seven membered lactam, six membered lactam **7.14** was obtained. This thesis proposes that this is due to the allyl amide functionality

forming a stable six membered catalyst deactivating chelate. This observation certainly warrants further investigation.

Methodology was developed for the stereoselective incorporation of a C-N constrained β-amino acid carbocycle, such as **7.29**, into a peptide or peptidomimetic. The application of this methodology to the synthesis of conformationally constrained calpain inhibitors is described in **Section 3.6** of this thesis.

References for Chapter 7

1. Nubbemeyer, U., Synthesis of medium-sized ring lactams. *Topics in Current Chemistry* **2001,** 216, 125-196.
2. Nathwani, D.; Wood, M. J., Penicillins: a current review of their clinical pharmacology and therapeutic use. *Drugs* **1993,** 45, (6), 866-94.
3. Schirmeister, T.; Klockow, A., Cysteine protease inhibitors containing small rings. *Mini reviews in medicinal chemistry* **2003,** 3, (6), 585-96.
4. Miller, S. J.; Blackwell, H. E.; Grubbs, R. H., Application of ring-closing metathesis to the synthesis of rigidified amino acids and peptides. *Journal of the American Chemical Society* **1996,** 118, (40), 9606-9614.
5. Abell, A. D.; Brown, K. M.; Coxon, J. M.; Jones, M. A.; Miyamoto, S.; Neffe, A. T.; Nikkel, J. M.; Stuart, B. G., Synthesis and evaluation of eight-membered cyclic pseudo-dipeptides. *Peptides* **2005,** 26, (2), 251-258.
6. Gardiner, J.; Abell, A. D., Synthesis and solid state conformation of phenylalanine mimetics constrained in a proline-like conformation. *Organic & Biomolecular Chemistry* **2004,** 2, (16), 2365-2370.
7. Gardiner, J.; Abell, A. D., A diastereoselective synthesis of the tetrahydropyridazinone core of 2-oxo-1,6-diazobicyclo[4.3.0]nonane-9-carboxylate-based peptidomimetics starting from (S)-phenylalanine. *Tetrahedron Letters* **2003,** 44, (22), 4227-4230.
8. Abell, A. D.; Gardiner, J., Synthesis of Lactam-Based Peptidomimetics from b-Keto Esters and b-Keto Amides. *Journal of Organic Chemistry* **1999,** 64, (26), 9668-9672.
9. Abell, A. D.; Gardiner, J.; Phillips, A. J.; Robinson, W. T., Synthesis and X-ray structure of a 1,2,3,6-tetrahydropyridine-based phenylalanine mimetic. *Tetrahedron Letters* **1998,** 39, (51), 9563-9566.
10. Abell, A. D.; Taylor, J. M.; Oldham, M. D., Self-addition products from the alkylation of amino acid-derived oxazolidinones: x-ray molecular structures of (2R,4S,1'S)-3-benzoyl-4-[benzoylamino(phenyl)methyl]-4-benzyl-2-phenyl-1,3-oxazolidin-5-one, (2R,4S,1'S)- and (2R,4S,1'R)-3-benzoyl-4-[benzoylamino(phenyl)methyl]-4-isopropyl-2-phenyl-1,3-oxazolidin-5-one. *Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry* **1996**, (11), 1299-1304.
11. Tikdari, A. M.; Fozooni, S., Reaction of 4-benzylidene-2-phenyloxazol-5(4H)-one with 3,4-dithio-toluene in the presence of triethylamine. *Heterocyclic Communications* **2003,** 9, (5), 473-478.

12. Formaggio, F.; Barazza, A.; Bertocco, A.; Toniolo, C.; Broxterman, Q. B.; Kaptein, B.; Brasola, E.; Pengo, P.; Pasquato, L.; Scrimin, P., Role of Secondary Structure in the Asymmetric Acylation Reaction Catalyzed by Peptides Based on Chiral Ca-Tetrasubstituted a-Amino Acids. *Journal of Organic Chemistry* **2004,** 69, (11), 3849-3856.
13. Gardiner, J. The synthesis of conformationally restricted amino acids and peptide mimics using ring closing metathesis. University of Canterbury, New Zealand, 2003.
14. Matthews, J. L.; Braun, C.; Guibourdenche, C.; Overhand, M.; Seebach, D., Preparation of enantiopure b-amino acids from a-amino acids using the Arndt-Eistert homologation. *Enantioselective Synthesis of b-Amino Acids* **1997**, 105-126.

8.1 General Methods and Experimental Procedures

Melting Points

All melting points were obtained on an Electrothermal apparatus and are uncalibrated.

Nuclear Magnetic Resonance

Proton NMR spectra were obtained on a Varian Inova spectrometer operating at either 300 MHz or 500 MHz. Carbon NMR spectra were obtained on a Varian Unity 300 spectrometer, operating at 75 MHz with a delay of (D_1) of 1s. All spectra were obtained at 23^o C unless specified. Chemical shifts are reported in parts per million (ppm) on the δ scale. Solvents used in NMR analysis (reference peak listed) included $CDCl_3$ (C**H**Cl_3 at δ_H 7.26 ppm, **C**DCl_3 at δ_C 77.0 ppm); CD_3OD (C**H**D_2OD at δ_H 3.31 ppm, **C**D_3OD at δ_C 49.05 ppm); $(CD_3)_2SO$ ($(CHD_2)_2SO$ at δ_H 2.50 ppm, $(CD_3)_2SO$ at δ_C 49.43 ppm).

All proton assignments were made on the basis of two-dimensional NMR experiments including COSY, HSQC, HMBC and nOe. All were obtained on the Varian Inova spectrometer operating at 500 MHz. The HSQC and HMBC experiments were all obtained with a delay of (D_1) of 1s.

Infrared Spectroscopy

Infrared spectra were obtained using a Shimadzu 9201PC series FTIR interfaced with an Intel 486 PC operating Shimadzu's HyperIR software. Spectra were obtained using solid KBr (diffuse reflectance).

Small Molecule Mass Spectrometry

Electron impact (EI) mass spectra were detected on a Kratos MS80 RFA mass spectrometer operating at 4000V (accelerating potential) and 70 eV (ionization energy).

The source temperature was 200-250^{o} C. Electrospray ionization (ESI) mass spectra were detected on a micromass LCT TOF mass spectrometer, with a probe voltage of 3200V, temperature of 150^{o} C and a source temperature of 80^{o} C. Direct ionization used 10µL of a 10µg mL^{-1} solution, using a carrier solvent of 50% acetonitrile/water at a flow rate of 20µL min^{-1}. Ionization was assisted by the addition of 0.5% formic acid.

Microanalysis

Microanyalysis was performed at the University of Otago Microanalytical Laboratory. All reported values are within ±0.4% of the calculated value.

Reagents, Solvents and Laboratory Methodology

Oven-dried glassware was used in all reactions performed under an inert atmosphere (either dry nitrogen or argon). All starting materials and reagents were obtained commercially unless otherwise stated.

Concentration *in-vacuo* refers to the removal of solvents "under reduced pressure" by rotary evaporation (low vacuum pump) followed by application of high vacuum (oil pump) for a minimum of thirty minutes.

Analytical thin layer chromatography (TLC) was performed on plastic-backed Merck Kieselgel KG60F$_{254}$ silica plates. Visualisation was achieved using short wave ultraviolet light and $KMNO_4$ or phosphomolybdate dip. Flash chromatography was performed using 230-400 mesh Merck Silica gel 60 either under a positive pressure of dry nitrogen or using the Buchi sepacore flash chromatography system.

THF and diethyl ether were distilled from sodium benzophenone ketyl under an inert atmosphere immediately prior to use. Dichloromethane, 1,2-dichloroethane, benzene, toluene, triethylamine and 1,1,2-trichloroethane were distilled from calcium hydride

under an inert atmosphere. Anhydrous DMF obtained from commercial sources. Petroleum ether refers to the fraction collected between 50-70^{o} C.

General Procedures

All general experimental procedures afforded a single stereoisomer as drawn unless noted otherwise.

General procedure A1: HATU mediated peptide coupling

The acid, amine (2 equiv) and HATU (1.1 equiv) were dissolved in anhydrous DMF (0.10–0.50M relative to acid). DIPEA was added (4 equiv) and the reaction mixture stirred at rt for 18 h. This was partitioned between ethyl acetate and 1M $HCl_{(aq)}$. The organic phase was then washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure A2: HATU mediated peptide coupling

The acid, amine (1.1 equiv) and HATU (1.1 equiv) were dissolved in anhydrous DMF (0.10–0.50M relative to acid). DIPEA was added (4 equiv) and the reaction mixture stirred at rt for 18 h. This was partitioned between ethyl acetate and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure A3: HATU mediated peptide coupling

The acid, amine (2 equiv), HATU (1.1 equiv) and HOAt (1.1 equiv) were dissolved in anhydrous DMF (0.10–0.50M relative to acid). DIPEA (4 equiv) was added and the reaction mixture stirred at rt for 18 h. This was partitioned between ethyl acetate and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure B1: EDC mediated peptide coupling

The acid, EDC (1.1 equiv), HOBt.H_2O (1.1 eq) and amine (1.5 equiv) were dissolved in anhydrous DMF (0.10–0.50M relative to acid). DIPEA was added (4.75 equiv) and the reaction mixture stirred at rt for 18 h. This was partitioned between ethyl acetate and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure B2: EDC mediated peptide coupling

The acid, EDC (1.2 equiv), HOBt.H_2O (1.2 eq) and amine (1.1 equiv) were dissolved in anhydrous DMF (0.10–0.50M relative to acid). DIPEA was added (4 equiv) and the reaction mixture stirred at rt for 18 h. This was partitioned between ethyl acetate and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure B3: EDC mediated peptide coupling

The acid, EDC (1.3 equiv), HOBt.H_2O (1.5 eq) and amine (1.1 equiv) were dissolved in anhydrous DCM (0.10–0.50M relative to acid). DIPEA was added (2.5 equiv) and the reaction mixture stirred at rt for 18 h. More DCM was added and this was partitioned with 3M $HCl_{(aq)}$. The organic phase was washed sequentially with 3M $HCl_{(aq)}$, water and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure C1: Ester hydrolysis with base

The ester was dissolved in THF and to this NaOH (1.5 equiv) pre-dissolved in water was added (THF:H_2O ratio - 3.5:1). The minimum amount of methanol was added to obtain a homogenous solution and stirring was continued at rt for 18 h. The reaction mixture was concentrated *in-vacuo,* the residue partitioned between EtOAc and 1M $HCl_{(aq)}$, and the aqueous phase extracted twice more with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in-vacuo.* See individual experiments for details.

General procedure C2: Ester hydrolysis with base

The ester was dissolved in a 3:1 mixture of methanol and water (0.1M). LiOH (5 equiv) was added and the mixture stirred at rt for 18 h. The reaction mixture was concentrated *in-vacuo* and the residue partitioned between EtOAc and 1M $HCl_{(aq)}$. The aqueous phase was extracted twice more with EtOAc and the combined organic extracts washed with brine, dried ($MgSO_4$), filtered and concentrated *in-vacuo.* See individual experiments for details.

General procedure D1: Ring closing metathesis at room temperature

The diene was dissolved in anhydrous DCM (0.01M) under an atmosphere of argon. Grubbs second generation catalyst (0.10 equiv) was added and the mixture stirred at rt for eighteen h before being concentrated *in-vacuo*. See individual experiments for details.

General procedure D2: Ring closing metathesis using thermal heating

The diene was dissolved in anhydrous 1,1,2-trichloroethane (0.01M) under an atmosphere of argon. Grubbs second generation catalyst (0.10 equiv) was added. The mixture was heated at reflux for one hour. Two further additions of Grubbs second generation catalyst (0.10 equiv) were added and after each the reaction mixture was subjected to one h and eighteen h of reflux respectively. This was then cooled and concentrated *in-vacuo*. See individual experiments for details.

General procedure E1: Ring closing metathesis using microwave heating

The diene was dissolved in anhydrous 1,1,2-trichloroethane (0.01M) under an atmosphere of argon. Grubbs second generation catalyst (0.10 equiv) was added. The mixture was heated at reflux in the microwave (1200 W) for 20 min. Two further additions of Grubbs second generation catalyst (0.10 equiv) were added and after each the reaction mixture was subjected to a further 20 min heating in the microwave. This was then cooled and concentrated *in-vacuo*. See individual experiments for details.

General procedure E2: Ring closing metathesis using microwave heating and chloro-dicyclohexyl borane additive

The diene was dissolved in anhydrous 1,1,2-trichloroethane (0.01M) under an atmosphere of argon. To this were added 1M chloro-dicyclohexyl borane in hexanes (0.10 equiv) and Grubbs second generation catalyst (0.10 equiv). The mixture was heated at reflux in the microwave (1200 W) for 20 min. Two further additions of Grubbs second generation catalyst (0.10 equiv) were added and after each the reaction mixture was subjected to a further 20 min heating in the microwave. The mixture was then cooled and concentrated *in-vacuo*. See individual experiments for details.

General procedure F1: Esterification with thionyl chloride and methanol

The carboxylic acid was suspended in methanol. The mixture was cooled in ice and 10% (v/v) thionyl chloride was added portionwise. The solution was stirred in ice for one h and then at rt for 18 h before being concentrated *in-vacuo*. See individual experiments for details.

General procedure F2: Esterification with thionyl chloride and methanol

The carboxylic acid was suspended in methanol. This was cooled in ice and 20% (v/v) thionyl chloride was added portionwise. The solution was stirred in ice for one h and then at rt for 18 h before being concentrated *in-vacuo*. See individual experiments for details.

General procedure G1: Hydrogenation of a carbon-carbon double bond

The olefin was dissolved in methanol and 20% (w/w) of 10% palladium on carbon catalyst was added. The mixture was subjected to hydrogenation at rt and atmospheric pressure for 18 h. The mixture was filtered through celite and concentrated *in-vacuo*. See individual experiments for details.

General procedure G2: Hydrogenation of a carbon-carbon double bond

The olefin was dissolved in a 1:1 mixture of methanol/ethyl acetate and 20% (w/w) of 10% palladium on carbon catalyst was added. The mixture was subjected to hydrogenation at rt and atmospheric pressure for 18 h. The mixture was filtered through celite and concentrated *in-vacuo*. See individual experiments for details.

General procedure G3: Hydrogenation of a carbon-carbon double bond

The olefin was dissolved in a 1:1 mixture of methanol and DCM and 20% (w/w) of 10% palladium on carbon catalyst was added. The mixture was subjected to hydrogenation at rt and atmospheric pressure for 18 h. The mixture was filtered through celite and concentrated *in-vacuo*. See individual experiments for details.

General procedure H1: N-CBZ protection of an amino acid

The amine was dissolved in anhydrous DMF. Benzyl chloroformate (1.5 equiv) and DIPEA (4 equiv) were added, and the mixture stirred at rt for 18 h. The reaction mixture was partitioned between chloroform and 1M $HCl_{(aq)}$. The aqueous phase was separated and extracted three times with chloroform. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure H2: N-CBZ protection of an amino acid

The amine was dissolved in anhydrous DMF. Benzyl chloroformate (1.5 equiv) and DIPEA (4 equiv) were added and the mixture stirred at rt for 18 h. The reaction mixture was partitioned between ethyl acetate and 1M $HCl_{(aq)}$, the aqueous phase was separated and extracted three more times with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

General procedure I1: N-BOC cleavage

The *N*-BOC protected compound in 4M HCl in 1,4-dioxane was stirred at rt for 18 h. The solution was then concentrated *in-vacuo*. See individual experiments for details.

General procedure I2: N-BOC cleavage

The *N*-BOC protected compound in 10% TFA, DCM (v/v) was stirred at rt for 18 h. The solution was then concentrated *in-vacuo*. See individual experiments for details.

General procedure I3: N-BOC cleavage

The *N*-BOC protected compound in 2M HCl in diethyl ether was stirred at rt for 18 h. The solution was then concentrated *in-vacuo.* See individual experiments for details.

General procedure I4: N-BOC cleavage

The *N*-BOC protected compound in diethyl ether was cooled in ice and saturated with gaseous hydrogen chloride. This was warmed to rt, stirred for 18 h and concentrated *in-vacuo*. See individual experiments for details.

General procedure I5: N-BOC cleavage

The *N*-BOC protected compound dissolved/suspended in methanol was cooled in ice and 10% (v/v) thionyl chloride was added portionwise. The solution was stirred in ice for one h and then at rt for 18 h before being concentrated *in-vacuo*. See individual experiments for details.

General procedure J: One pot esterification and N-BOC cleavage

A suspension of *N*-BOC carboxylic acid in methanol was cooled in ice and 20% (v/v) thionyl chloride was added portionwise. The solution was stirred in ice for one h and then at rt for 18 h before being concentrated *in-vacuo*. See individual experiments for details.

General procedure K: N-BOC protection of an amino acid

The amino acid was dissolved in a 1/1 biphasic mixture of water and 1,4-dioxane. To this were added triethylamine (2.8 equiv) and di-tert-butyl dicarbonate (1.2 equiv). The mixture was stirred at rt for eighteen h before being concentrated *in vacuo*. The residue was partitioned between ethyl acetate and 10% (w/w) citric $acid_{(aq)}$. The aqueous phase was re-extracted twice more with ethyl acetate and the combined

organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure L: Sulfur trioxide/pyridine complex oxidation of an alcohol.

A solution of the alcohol in a 2:1 mixture of DMSO and DCM (0.10M) was cooled in ice and DIPEA (4 equiv) was added. To this a solution of SO_3.Pyr complex dissolved in DMSO was added. The mixture was stirred in ice for one h (or until TLC indicated complete consumption of the starting alcohol). The reaction mixture was diluted with ethyl acetate and partitioned with 1M $HCl_{(aq)}$. The organic phase was washed with saturated $NaHCO_{3(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure M1: Sulfonyl chloride coupling to an amine.

The amine was dissolved in anhydrous DCM (0.1M) under an atmosphere of argon. To this were added anhydrous triethylamine (4 equiv) and the sulfonyl chloride (1.1 equiv). The mixture was stirred at rt for eighteen h. The reaction mixture was diluted with further DCM and partitioned with 1M $HCl_{(aq)}$. The organic phase was washed twice more with 1M $HCl_{(aq)}$, twice with 50% $NaHCO_{3(aq)}$ and once with brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure M2: Sulfonyl chloride coupling to an amine.

To a solution of the amine in anhydrous DCM (0.1M) under an atmosphere of argon, were added DIPEA (2.2 equiv) and sulfonyl chloride (1.1 equiv). The mixture was stirred at rt for eighteen h before concentrated *in vacuo*. The residue was partitioned between ethyl acetate and 1M $HCl_{(aq)}$ and the organic phase washed sequentially with 1M $HCl_{(aq)}$ and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure M3: Sulfonyl chloride coupling to an amine.

To a solution of the amine in anhydrous DMF (0.1M) under an atmosphere of argon, were added DIPEA (2.2 equiv) and sulfonyl chloride (1.1 equiv). The mixture was stirred at rt for eighteen h. The reaction mixture was diluted with ethyl acetate and partitioned with 1M $HCl_{(aq)}$. The organic phase was washed again with 1M $HCl_{(aq)}$ and then with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure O1: N-allylation.

To a solution of the amine in anhydrous DMF (0.2M) under an atmosphere of argon, were added potassium carbonate (2 equiv) and allyl bromide (1.2 equiv). The mixture was stirred at rt for eighteen h before being diluted with ethyl acetate and partitioned with 1M $HCl_{(aq)}$. The aqueous phase was extracted twice more with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure O2: N-allylation.

To a solution of the amine in anhydrous DMF (0.2M) under an atmosphere of argon, were added potassium carbonate (2 equiv) and allyl bromide (4 equiv). The mixture was stirred at rt for eighteen h before being diluted with ethyl acetate and partitioned with 1M $HCl_{(aq)}$. The aqueous phase was extracted twice more with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure O3: N-allylation.

The sulfonamide/amide was dissolved in anhydrous THF (0.1M) under an atmosphere of argon. The solution was cooled to -78^{o}C and P_4-phosphazene (2 equiv per NH) was added. The mixture was stirred at -78^{o}C for one h and then allyl bromide (2 equiv per NH) was added. The mixture was stirred at -78^{o}C for a further two h and then at rt for eighteen h before being concentrated *in vacuo*. The residue was partitioned between ethyl acetate and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure P: Arndt-Eistert homologation.

The *N*-protected α-amino acid was dissolved in anhydrous THF, under an atmosphere of argon and this was cooled to -25^{o}C. Triethylamine (1 equiv) and ethyl chloroformate (1 equiv) were added and the mixture was stirred at -25^{o}C for twenty min. The mixture was warmed in ice and ethereal diazomethane (~1.2 equiv) was added dropwise over ten min. This was stirred in ice for two h and then at rt for a further sixteen h. Glacial acetic acid was added to quench the reaction followed by 1M $NaOH_{(aq)}$ to adjust the solution to pH 8. Ethyl acetate and water were added and the mixture allowed to partition. The aqueous phase was separated and extracted again with ethyl acetate. The combined organic extracts were washed sequentially with saturated $NH_4Cl_{(aq)}$ and brine, before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resultant diazoketone was used without purification. A suspension the diazoketone in super-

dry methanol (0.25M), under an atmosphere of argon, was cooled to -25°C and a solution of silver benzoate (0.11 equiv) in triethylamine (2.9 equiv) was added. The mixture was stirred at -25°C for fifteen min and then at rt for eighteen h before being concentrated *in vacuo*. The residue was partitioned between ethyl acetate and saturated $NaHCO_{3(aq)}$. The organic phase was washed sequentially with saturated $NH_4Cl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure Q: C-allylation.

A solution of diisopropylamine (2.2 equiv) and LiCl (3 equiv) in anhydrous THF (0.1M) under an atmosphere of argon, was cooled to -78°C. ^{n}BuLi (2.2 equiv) was added and the mixture was stirred at -78°C for thirty min. A solution of the *N*-protected β-amino acid (1 equiv) in THF (1M) and allyl bromide (4 equiv) were added. Stirring was continued at -78°C for one h and then at rt for a further seventeen h before being concentrated *in vacuo*. The residue was partitioned between ethyl acetate and saturated $NH_4Cl_{(aq)}$, the organic phase washed with saturated $NaHCO_{3(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

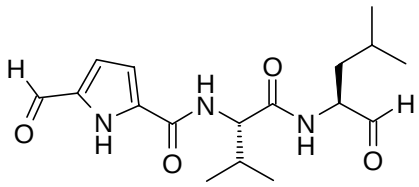
General procedure R: DIBAL-H reduction of methyl ester to aldehyde.

A solution of the ester in anhydrous DCM (0.01M), under an atmosphere of argon, was cooled to -78 ˚C. DIBAL-H (5.5 equiv) was added dropwise. The mixture was stirred at -78 ˚C for three h and super-dry methanol (1:1 ratio with DCM initially added), precooled to -78 ˚C, was added dropwise and the mixture stirred at -78 ˚C for a further twenty five min. The cooling bath was removed and 1M $HCl_{(aq)}$ added. The organic layer was separated from the resulting white precipitate, diluted with ethyl acetate and allowed to partition. The organic phase was washed sequentially with 1M $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. See individual experiments for details.

General procedure S: Lithium aluminium hydride reduction of methyl ester to alcohol.

A solution of the methyl ester dissolved in anhydrous THF (0.1M), under an atmosphere of argon, was cooled in ice. 1M $LiAlH_4$ in either diethyl ether or THF was added (1.1 equiv). The mixture was stirred in ice for one h and then at rt for a further seventeen h. Methanol was added to quench the reaction and the mixture was stirred at rt for ten min before being concentrated *in-vacuo*. The residue was partitioned between EtOAc and 1M $KHSO_{4(aq)}$. The aqueous phase was extracted twice more with chloroform and each organic extract was washed with brine before being combined, dried ($MgSO_4$), filtered and concentrated *in-vacuo*. See individual experiments for details.

5-Formyl-1H-pyrrole-2-carboxylic acid [1-((S)-(S)-1-formyl-3-methyl-butylcarbamoyl)-2-methylpropyl]-amide (2.13)



Alcohol **2.18** (0.600g, 1.78 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.394g, 66%. R_f = 0.38 (2/1 (EtOAc / (50/70) Pet ether)).

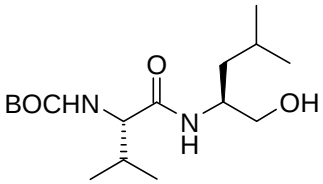
^{1}H NMR ppm (500 MHz in $CDCl_3$) 11.71 (1H, bs, N**H** Pyrrole), 9.61 (1H, s, C**H**O), 9.59 (1H, s, C**H**O), 7.55 (1H, d J=6.7Hz, N**H** Leu), 7.35 (1H, d J=8.4Hz, N**H** Val), 6.96 (1H, d J=2.7Hz, Ar-**H**), 6.79 (1H, d J=2.7Hz, Ar-**H**), 4.79 (1H, dd, J=8.4Hz, J=8.4Hz, C**H**CH$(CH_3)_2$), 4.44-4.58 (1H, m, C**H**CH_2CH$(CH_3)_2$), 2.07-2.19 (1H, m, CHC**H**$(CH_3)_2$), 1.60-1.69 (2H, m, CHC**H$_2$**CH$(CH_3)_2$), 1.33-1.48 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.07 (3H, d J=6.6 Hz, CHCH(C**H$_3$**)$_2$), 1.02 (3H, d, J=6.6 Hz, CHCH(C**H$_3$**)$_2$), 0.85 (3H, d J=6.1 Hz, $CHCH_2$CH(C**H$_3$**)$_2$), 0.82 (3H, d J=6.1 Hz, $CHCH_2$CH(C**H$_3$**)$_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 199.4, 180.5, 172.1, 160.0, 134.2 , 132.0, 121.0, 111.4, 58.9, 57.5, 37.3, 31.0, 24.6, 22.8, 21.7, 19.2, 18.8

HRMS (ES) 336.1925 (MH^+). $C_{17}H_{25}N_3O_4$ requires 336.1923

Microanalysis. C, 58.99; H, 7.28; N, 12.07. $C_{17}H_{25}N_3O_4$ requires C, 59.29; H, 7.61; N, 12.20

[(S)-1-((S)-1-Hydroxymethyl-3-methyl-butylcarbamoyl)-2-methylpropyl]carbamic acid tert-butyl ester (2.14)



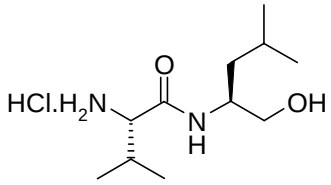
N-BOC-Val-H (9.26g, 42.7 mmol) was reacted with (L)-leucinol using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 9.18g, 68%. R_f = 0.24 (2/1 (EtOAc / (50/70) Pet ether)).
mp 54-56 ºC

^{1}H NMR ppm (500 MHz in $CDCl_3$) 6.24 (1H, d J=7.9Hz, N**H** Leu), 5.10 (1H, d J=7.32Hz, N**H** Val), 3.99–4.06 (1H, m, C**H**CH_2CH$(CH_3)_2$), 3.80 (1H, dd J=7.3Hz, J=7.3Hz, C**H**CH$(CH_3)_2$), 3.65 (1H, dd J=3.2Hz, J=11.1Hz, C**H$_2$**OH), 3.50 (1H, dd J=5.6Hz, J=11.1Hz, C**H$_2$**OH), 2.06-2.16 (1H, m, CHC**H**$(CH_3)_2$), 1.58-1.62 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.43 (9H, s, C(C**H$_3$**)$_3$), 0.88-0.97 (12H, m, $CHCH_2$CH(C**H$_3$**)$_2$ and CHCH(C**H$_3$**)$_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 172.9, 163.3, 79.2, 64.3, 60.7, 49.3, 39.8, 30.4, 27.4, 24.4, 22.6, 21.0, 18.5, 17.3

HRMS (ES) 317.2249 (MH^+). $C_{16}H_{32}N_2O_4$ requires 317.2240

(S)-2-Amino-N-((S)-1-hydroxymethyl-3-methylbutyl)-3-methylbutyramide (2.15)



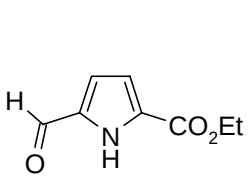
N-BOC protected compound **2.14** (7.00g, 22.2 mmol) was reacted using General Procedure I1 to afford a white solid, 5.59g, 100%.
mp 64-66 ºC

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 8.13 (2H, bs, N**H$_2$**) 4.72-4.77 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.79-3.87 (1H, m, C**H**$CH(CH_3)_2$) 3.52-3.56 (1H, m, C**H$_2$**OH), 3.26 (1H, dd J=6.0Hz, J=16.0Hz, C**H$_2$**OH), 2.02-2.08 (1H, m, CHC**H**$(CH_3)_2$), 1.60-1.68 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.30-1.33 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 0.85-0.94 (12H, m, $CHCH_2CH(CH_3)_2$ and $CHCH(CH_3)_2$)

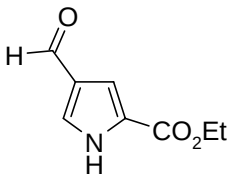
^{13}C NMR ppm (75 MHz in $(CD_3)_2SO$) 167.1, 63.4, 57.3, 49.2, 29.7, 23.9, 23.3, 21.9, 18.2, 17.9.

HRMS (ES) 217.1910 (MH^+). $C_{11}H_{24}N_2O_2$ requires 217.1916

5-Formyl-1H-pyrrole-2-carboxylic acid ethyl ester (2.16) and 4-Formyl-1H-pyrrole-2-carboxylic acid ethyl ester (2.19)



2.16



2.19

Phosphorus oxychloride (7.36 mL, 79.0 mmol) was added dropwise over fifteen min to DMF (1 equiv) in ice under an atmosphere of argon. A solution of pyrrole-2-carboxylic ethyl ester (0.91 equiv) in DCE (18 mL) was added dropwise at in ice over one h. This was then heated at reflux for fifteen min, cooled in ice and a solution of sodium acetate tri-hydrate (5.00 equiv) in water (80 mL) was added. This was refluxed for fifteen min, cooled and diethyl ether (150 mL) was added. The mixture was partitioned and the aqueous phase was extracted twice more with diethyl ether. The combined organic extracts were washed sequentially with saturated $NaHCO_{3(aq)}$ and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield 5-formyl product as a yellow solid, 6.25g, 52%. R_f = 0.46 (1/1 (EtOAc / (50/70) Pet ether)) and 4-formyl product as a brown solid, 3.24g, 27%. R_f = 0.29 (1/1 (EtOAc / (50/70) Pet ether)).

5-Formyl-1H-pyrrole-2-carboxylic acid ethyl ester (2.16) Lit[1]

mp 71-73°c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.95 (1H, bs, N**H**), 9.66 (1H, s, C**H**O), 6.92-6.94 (2H, m, Ar-**H**), 4.38 (2H, q J=7.1Hz, $CO_2C\mathbf{H_2}CH_3$), 1.38 (3H, t J=7.1Hz, $CO_2CH_2C\mathbf{H_3}$)

LRMS (ES) 168.1 (MH^+). $C_8H_9NO_3$ requires 168.1

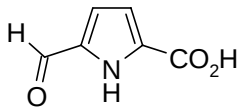
4-Formyl-1H-pyrrole-2-carboxylic acid ethyl ester (2.19) Lit[2]

mp 102-104°c. Lit[2] 104-106°c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 10.32 (1H, bs, N**H**), 9.84 (1H, s, C**H**O), 7.58 (1H, d J=1.5Hz, Ar-**H**), 7.32 (1H, d J=1.5Hz, Ar-**H**), 4.35 (2H, q J=7.1Hz, J=7.1Hz, J=7.2Hz, $CO_2C\mathbf{H_2}CH_3$), 1.37 (3H, t, J=7.1Hz, J=7.1Hz, $CO_2CH_2C\mathbf{H_3}$)

LRMS (ES) 168.1 (MH^+). $C_8H_9NO_3$ requires 168.1

5-Formyl-1H-pyrrole-2-carboxylic acid (2.17) Lit[1]



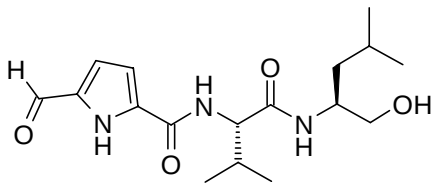
To a suspension of ethyl ester **2.16** (6.30g, 37.7 mmol) in water (190 mL) potassium hydroxide (4 equiv) was added. This was stirred at 40°C for two h, cooled, acidified to pH 1 using $_c$HCl and EtOAc (200 mL) added. This was partitioned and the aqueous phase extracted twice more with EtOAc. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a yellow solid, 5.03g, 96%.
mp 200-203°c.

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 13.09 (1H, bs, $CO_2\mathbf{H}$), 12.88 (1H, bs, N**H**), 9.68 (1H, s, C**H**O), 6.94 (1H, d J=3.7Hz, Ar-**H**), 6.83 (1H, d J=3.7Hz, Ar-**H**))

^{13}C NMR ppm (75 MHz in $(CD_3)_2SO$). 176.1, 156.2, 130.0, 123.8, 111.2, 110.2

LRMS (ES) 140.0 (MH^+). $C_6H_5NO_3$ requires 140.0

5-Formyl-1H-pyrrole-2-carboxylic acid {1-[(S)-1-((S)-hydroxymethyl)-3-methylbutylcarbamoyl]-2-methyl-propyl}amide (2.18)



Carboxylic acid **2.17** (0.321g, 2.31 mmol) was reacted with amine **2.15** using General Procedure B3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.304g, 39%. R_f = 0.22 (2/1 (EtOAc / (50/70) Pet ether)).

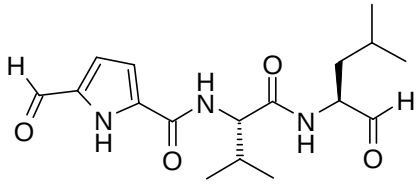
^{1}H NMR ppm (500 MHz in CD_3OD) 9.58 (1H, s, C**H**O), 6.99 (1H, d J=4.0Hz, Ar-**H**), 6.95 (1H, d J=4.0Hz, Ar-**H**), 4.28 (1H, d J=8.3 Hz, C**H**CH$(CH_3)_2$), 3.95-4.02 (1H, m, C**H**CH_2CH$(CH_3)_2$), 3.47 (1H, dd J=5.4Hz, J=10.9Hz, C**H$_2$**OH), 3.43 (1H, dd J=5.6Hz, J=10.9, C**H$_2$**OH), 2.08-2.17 (1H, m, CHC**H**$(CH_3)_2$), 1.58-1.69 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.31-1.34 (2H, m, CHC**H$_2$**CH$(CH_3)_2$), 0.99 (3H, d J=6.7 Hz, CHCH(C**H$_3$**$)_2$), 0.98 (3H, d J=6.7 Hz, CHCH(C**H$_3$**$)_2$), 0.88 (3H, d J=6.7Hz, $CHCH_2$CH(C**H$_3$**$)_2$), 0.84 (3H, d J=6.7 Hz, $CHCH_2$CH(C**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in CD_3OD). 180.9, 172.1, 134.6, 119.2, 112.5, 112.0, 64.2, 59.5, 49.4, 39.7, 30.4, 24.5, 22.4, 20.9, 18.5, 17.8

HRMS (ES) 338.2080 (MH^+). $C_{17}H_{27}N_3O_4$ requires 338.2086

Microanalysis. C, 59.89; H, 8.44; N, 13.70. $C_{17}H_{27}N_3O_4$ requires C, 60.35; H, 8.86; N, 13.20

5-Formyl-1H-pyrrole-2-carboxylic acid [1-((S)-(S)-1-formyl-3-methylbutylcarbamoyl)-2-methylpropyl]-amide (2.13)



Alcohol **2.18** (0.600g, 1.78 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.394g, 66%. R_f = 0.38 (2/1 (EtOAc / (50/70) Pet ether)).

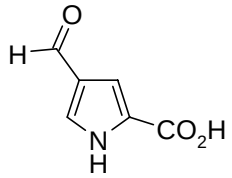
^{1}H NMR ppm (500 MHz in $CDCl_3$) 11.71 (1H, bs, N**H** Pyrrole), 9.61 (1H, s, C**H**O), 9.59 (1H, s, C**H**O), 7.55 (1H, d J=6.7Hz, N**H** Leu), 7.35 (1H, d J=8.4Hz, N**H** Val), 6.96 (1H, d J=2.7Hz, Ar-**H**), 6.79 (1H, d J=2.7Hz, Ar-**H**), 4.79 (1H, dd, J=8.4Hz, J=8.4Hz, C**H**CH$(CH_3)_2$), 4.44-4.58 (1H, m, C**H**CH_2CH$(CH_3)_2$), 2.07-2.19 (1H, m, CHC**H**$(CH_3)_2$), 1.60-1.69 (2H, m, CHC**H$_2$**CH$(CH_3)_2$), 1.33-1.48 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.07 (3H, d J=6.6 Hz, CHCH(C**H$_3$**$)_2$), 1.02 (3H, d, J=6.6 Hz, CHCH(C**H$_3$**$)_2$), 0.85 (3H, d J=6.1 Hz, $CHCH_2$CH(C**H$_3$**$)_2$), 0.82 (3H, d J=6.1 Hz, $CHCH_2$CH(C**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 199.4, 180.5, 172.1, 160.0, 134.2 , 132.0, 121.0, 111.4, 58.9, 57.5, 37.3, 31.0, 24.6, 22.8, 21.7, 19.2, 18.8

HRMS (ES) 336.1925 (MH^+). $C_{17}H_{25}N_3O_4$ requires 336.1923

Microanalysis. C, 58.99; H, 7.28; N, 12.07. $C_{17}H_{25}N_3O_4$ requires C, 59.29; H, 7.61; N, 12.20

4-Formyl-1H-pyrrole-2-carboxylic acid (2.20) Lit[2]



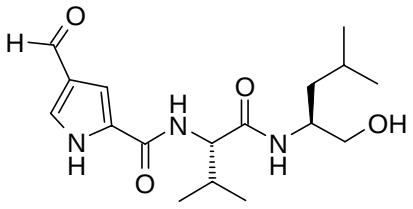
To a suspension of ethyl ester **2.19** (3.20g, 19.1 mmol) in water (100 mL) was added potassium hydroxide (4 equiv). This was stirred at 40^{o}C for two h, cooled, acidified to pH 1 using $_c$HCl and EtOAc added (200 mL). This was partitioned and the aqueous phase extracted twice more with EtOAc. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a brown solid, 2.50g, 94%.

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 12.85 (1H, bs, CO_2**H**), 12.53 (1H, s, N**H**), 9.74 (1H, s, C**H**O), 7.77 (1H, d J=1.5Hz, Ar-**H**), 7.07 (1H, d J=1.5Hz, Ar-**H**)

^{13}C NMR ppm (75 MHz in $(CD_3)_2SO$). 185.8, 161.5, 130.8, 126.5, 125.6, 112.5

LRMS (ES) 140.0 (MH^+). $C_6H_5NO_3$ requires 140.0

4-Formyl-1H-pyrrole-2-carboxylic acid {1-[(S)-1-((S)-hydroxymethyl)-3-methylbutylcarbamoyl]-2-methyl-propyl}amide (2.21)



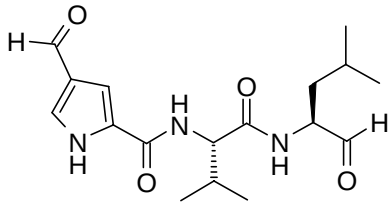
Carboxylic acid **2.20** (0.175g, 1.26 mmol) was reacted with amine **2.15** using General Procedure B3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.102g, 24%. R_f = 0.18 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 11.49 (1H, bs, N**H** Pyrrole), 9.80 (1H, s, C**H**O), 7.57-7.70 (4H, m, Ar-**H** and N**H** Leu and N**H** Val), 4.56 (1H, dd J=8.8Hz, J=8.8Hz, C**H**CH$(CH_3)_2$), 4.07-4.16 (1H, m, C**H**CH_2CH$(CH_3)_2$), 3.75 (1H, dd J=3.3Hz, J=11.2Hz, C**H$_2$**OH), 3.63 (1H, dd J=5.6Hz, J=11.2Hz, C**H$_2$**OH), 2.11-2.19 (1H, m, CHC**H**$(CH_3)_2$,), 1.52-1.68 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.26-1.44 (2H, m, CHC**H$_2$**CH$(CH_3)_2$), 1.01 (3H, d J=6.7Hz, CHCH(C**H$_3$**)$_2$), 0.97 (3H, d J=6.7 Hz, CHCH(C**H$_3$**)$_2$), 0.82 (3H, d J=6.5Hz, $CHCH_2$CH(C**H$_3$**)$_2$), 0.77 (3H, d J=6.5Hz, $CHCH_2$CH(C**H$_3$**)$_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.4, 160.9, 130.1, 128.1, 127.0, 109.2, 65.7, 59.4, 50.1, 39.7, 31.2, 24.8, 22.7, 22.2, 19.2, 18.8

HRMS (ES) 338.2079 (MH^+). $C_{17}H_{27}N_3O_4$ requires 338.2086

4-Formyl-1H-pyrrole-2-carboxylic acid [1-((S)-(S)-1-formyl-3-methylbutylcarbamoyl)-2-methylpropyl]-amide (2.22)

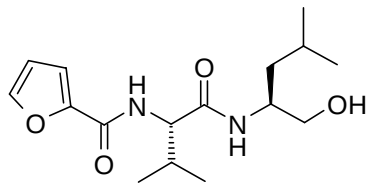


Alcohol **2.21** (0.100g, 0.296 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.0179g, 18%. R_f = 0.26 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 10.01 (1H, bs, N**H** Pyrrole), 9.85 (1H, s, C**H**O), 9.84 (1H, s, C**H**O), 7.59-7.61 (2H, m, N**H** Leu and N**H** Val), 7.56-7.59 (2H, m, Ar-**H**), 4.28-4.43 (2H, m, C**H**$CH(CH_3)_2$ and C**H**$CH_2CH(CH_3)_2$), 2.03-2.22 (1H, m, CHC**H**$(CH_3)_2$), 1.35-1.43 (3H, m $CHCH_2$C**H**$(CH_3)_2$ and CHC**H$_2$**$CH(CH_3)_2$), 1.01 (3H, d J=6.5Hz, $CHCH(CH_3)_2$), 0.98 (3H, d J=6.5 Hz, $CHCH(CH_3)_2$), 0.84 (3H, d J=6.4Hz, $CHCH_2CH(CH_3)_2$), 0.79 (3H, d J=6.4Hz, $CHCH_2CH(CH_3)_2$)

HRMS (ES) 336.1918 (MH^+). $C_{17}H_{25}N_3O_4$ requires 336.1923

Furan-2-carboxylic acid [(S)-1-((S)-1-hydroxymethyl-3-methylbutylcarbamoyl)-2-methylpropyl]amide (2.24)



Amino alcohol **2.15** (1.00g, 3.96 mmol) was dissolved in anhydrous DCM (30 mL) under at atmosphere of argon. To this DMAP (1.1 equiv) and triethylamine (3 equiv) were added. This was cooled in ice and 2-furoyl chloride added (1 equiv). This was stirred in ice for two h and then at rt for eighteen h. The reaction mixture was diluted with DCM, partitioned with 1M $HCl_{(aq)}$ and the organic phase dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.344g, 28%. R_f = 0.11 (1/1 (EtOAc / (50/70) Pet ether)).
mp 38-4in ice.

^{1}H NMR ppm (500 MHz in CD_3OD) 7.66 (1H, dd J=1.2Hz, J=2.1Hz, Ar-**H**), 7.13 (1H, dd J=2.1Hz, J=3.4Hz, Ar-**H**), 6.57 (1H, dd J=1.2Hz, J=3.4Hz, Ar-**H**), 4.29 (1H, d J=7.9Hz, C**H**$CH(CH_3)_2$), 3.97-4.01 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.51 (1H, dd J=5.3Hz, J=10.8Hz, C**H$_2$**OH), 3.46 (1H, dd J=5.6Hz, J=10.8Hz, C**H$_2$**OH), 2.10-2.19 (1H, m, CHC**H**$(CH_3)_2$), 1.58-1.67 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.31-1.42 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 1.00 (3H, d J=6.8 Hz, $CHCH(CH_3)_2$), 0.98 (3H, d J=6.8 Hz, $CHCH(CH_3)_2$), 0.87 (3H, d J=6.7 Hz, $CHCH_2CH(CH_3)_2$), 0.85 (3H, d J=6.7Hz, $CHCH_2CH(CH_3)_2$)

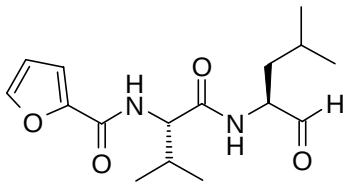
^{13}C NMR ppm (75 MHz in CD_3OD). 172.0, 159.0, 147.2, 145.1, 114.4, 111.8, 64.3, 59.0, 49.5, 39.7, 30.8, 24.6, 22.5, 21.1, 18.6, 17.8

HRMS (ES) 311.1793 (MH^+). $C_{16}H_{26}N_2O_4$ requires 311.1971

FTIR CM^{-1} (KBr) 3277, 2958, 1633, 1593, 1519, 758

Microanalysis. C, 61.83; H, 8.93; N, 8.84. $C_{16}H_{26}N_2O_4$ requires C, 61.91; H, 8.44; N, 9.03

Furan-2-carboxylic acid [(S)-1-((S)-1-formyl-3-methylbutylcarbamoyl)-2-methylpropyl]amide (2.25)



Alcohol **2.24** (0.340g, 1.10 mmol) was oxidised using General Procedure L to afford a white solid, 0.304g, 90%. mp 42-44^{o}c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.52 (1H, s, C**H**O), 7.59 (1H, d J=7.4Hz, N**H** Leu), 7.39 (1H, bs, Ar-**H**), 7.15 (1H, d J=9.1Hz, N**H** Val), 7.07 (1H, dd J=0.8Hz, J=3.2Hz, Ar-**H**), 6.42 (1H, dd J=1.4Hz, J=3.2Hz, Ar-**H**), 4.68 (1H, dd J=7.4Hz, J=9.1Hz, C**H**$CH(CH_3)_2$), 4.38-4.42 (1H, m, C**H**$CH_2CH(CH_3)_2$), 2.15-2.23 (1H, m, CHC**H**$(CH_3)_2$), 1.55-1.61 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 1.35-1.44 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.04 (3H, d J=6.7Hz, $CHCH(CH_3)_2$), 1.00 (3H, d J=6.7 Hz, $CHCH(CH_3)_2$), 0.83 (3H, d J=5.5 Hz, $CHCH_2CH(CH_3)_2$), 0.80 (3H, d J=5.5Hz, $CHCH_2CH(CH_3)_2$)

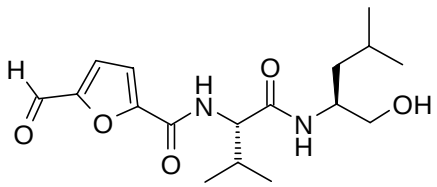
^{13}C NMR ppm (75 MHz in $CDCl_3$) 199.6, 171.6, 158.2, 147.2, 144.4, 114.7, 112.0, 57.7, 57.2, 37.1, 31.6, 24.6, 22.8,21.7, 19.2, 18.2

HRMS (ES) 309.1820 (MH^+). $C_{16}H_{24}N_2O_4$ requires 309.1814

FTIR CM^{-1}(KBr) 3287, 2961, 1738, 1645, 1519, 758

Microanalysis. C, 58.50; H, 7.73; N, 8.47. $C_{16}H_{26}N_2O_4$ requires C, 58.88; H, 8.03; N, 8.58

5-Formylfuran-2-carboxylic acid {1-[(S)-1-((S)-hydroxymethyl)-3-methylbutylcarbamoyl]-2-methyl-propyl}amide (2.27)



5-Formyl-furyl-2-carboxylic acid (0.554g, 3.96 mmol) was reacted with amine **2.15** using General Procedure B2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.321g, 24%. R_f = 0.43 (2/1 (EtOAc / (50/70) Pet ether)).
mp 46-48^{o}c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.75 (1H, s, C**H**O), 7.25-7.29 (2H, m, Ar-**H**), 7.26 (1H, d J=6.4Hz N**H** Val), 6.21 (1H, d J=8.0Hz, N**H** Leu), 4.38-4.41 (1H, m, C**H**CH$(CH_3)_2$), 4.05-4.10 (1H, m, C**H**CH_2CH$(CH_3)_2$), 3.71 (1H, dd J=3.4Hz, J=11.0Hz, C**H$_2$**OH), 3.60 (1H, dd J=5.5Hz, J=11.0Hz, C**H$_2$**OH), 2.17-2.26 (1H, m, CHC**H**$(CH_3)_2$), 1.51-1.60 (1H, m, CHCH_2C**H**$(CH_3)_2$), 1.37-1.43 (2H, m, CHC**H$_2$**CH$(CH_3)_2$), 0.97 (3H, d J=7.2Hz, CHCH(C**H$_3$**)$_2$), 0.96 (3H, d J=7.2 Hz, CHCH(C**H$_3$**)$_2$), 0.81 (3H, d J=5.7Hz, CHCH_2CH(C**H$_3$**)$_2$), 0.80 (3H, d J=5.7 Hz, CHCH_2CH(C**H$_3$**)$_2$)

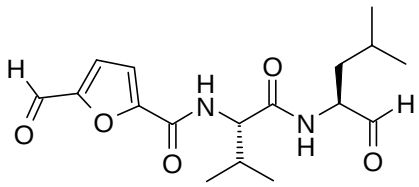
^{13}C NMR ppm (75 MHz in $CDCl_3$) 178.5, 170.9, 170.8, 157.3, 152.5, 150.6, 115.9, 65.1, 58.8, 49.9, 39.8, 31.5, 24.8, 22.8, 22.3, 19.2, 18.5

HRMS (ES) 339.1906 (MH^+). $C_{17}H_{26}N_2O_5$ requires 339.1920

FTIR CM^{-1} (KBr) 2962, 2353, 1688, 1655, 1543

Microanalysis. C, 60.58; H, 7.57; N, 8.09. $C_{17}H_{26}N_2O_5$ requires C, 60.34; H, 7.74; N, 8.28

5-Formylfuran-2-carboxylic acid [1-((S)-(S)-1-formyl-3-methylbutylcarbamoyl)-2-methylpropyl] amide (2.28)



Alcohol **2.27** (0.180g, 0.532 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.100g, 56%. R_f = 0.42 (2/1 (EtOAc / (50/70) Pet ether)).
mp 39-41^{o}c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.73 (1H, s, Ar-C**H**O), 9.59 (1H, s, Leu-C**H**O), 7.35 (1H, d J=8.8Hz, N**H** Val), 7.30 (1H, d J=3.6Hz, Ar-**H**), 7.26 (1H, d J=3.6Hz, Ar-**H**), 6.85 (1H, d J=7.1Hz, N**H** Leu), 4.54-4.60 (2H, m, C**H**CH$(CH_3)_2$ and C**H**$CH_2CH(CH_3)_2$), 2.20-2.28 (1H, m, CHC**H**$(CH_3)_2$), 1.66-1.72 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 1.40-1.47 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.06 (3H, d J=6.9 Hz, CHCH(C**H$_3$**$)_2$), 1.04 (3H, d J=6.9 Hz, CHCH(C**H$_3$**$)_2$), 0.93 (3H, d J= 5.6 Hz, $CHCH_2$CH(C**H$_3$**$)_2$), 0.92 (3H, d J=5.6 Hz, $CHCH_2$CH(C**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 199.6, 178.5, 171.2, 157.4, 152.6, 150.5, 121.1, 116.0, 58.4, 57.4, 37.3, 31.4, 24.7, 22.9, 21.8, 19.3, 18.4

HRMS (ES) 337.1761 (MH^+). $C_{17}H_{24}N_2O_5$ requires 337.1763

FTIR CM^{-1} (KBr) 3279, 2959, 1734, 1687, 1651, 1539, 1494

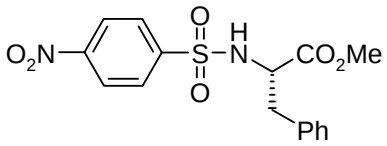
Microanalysis. C, 60.37; H, 7.35; N, 8.09. $C_{17}H_{26}N_2O_5$ requires C, 60.70; H, 7.19; N, 8.33

References for Chapter 2 Experimental

1. Martyn, D. C.; Vernall, A. J.; Clark, B. M.; Abell, A. D., Ring-deactivated hydroxyalkylpyrrole-based inhibitors of alpha-chymotrypsin: synthesis and mechanism of action. *Organic & Biomolecular Chemistry* **2003,** 1, (12), 2103-2110.

2. Garrido, D. O. A.; Buldain, G.; Ojea, M. I.; Frydman, B., Synthesis of 2-alkylputrescines from 3-alkylpyrroles. *Journal of Organic Chemistry* **1988,** 53, (2), 403-7.

(S)-2-(4-Nitrobenzenesulfonylamino)-3-phenylpropionic acid methyl ester (3.21) Lit[1]

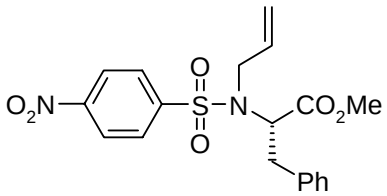


Phe-OMe (3.34g, 15.3 mmol) was reacted with 4-nitrobenzene sulfonyl chloride using General Procedure M1 to afford a white solid, 4.57g, 81%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.22 (2H, d J=8.7Hz, Ar-**H** (4-NO_2 Ph)), 7.84 (2H, d J=8.7Hz, Ar-**H** (4-NO_2 Ph)), 7.21-7.23 (3H, m, Ar-**H** (Phe)), 7.05 (2H, dd J=2.6Hz, J=5.9Hz, Ar-**H** (Phe)), 5.28 (1H, d J=9.3Hz, N**H**), 4.26 (1H, ddd J=5.5Hz, J=7.0Hz, J=9.3Hz, C**H**CO_2CH_3), 3.62 (3H, s, $CO_2C\mathbf{H_3}$), 3.12 (1H, dd J=5.5Hz, J=13.9Hz, CHC$\mathbf{H_2}$Ph), 2.98 (1H, dd J=7.0Hz, J=13.9Hz, CHC$\mathbf{H_2}$Ph)

LRMS (ES) 365.1 (MH^+). $C_{16}H_{16}N_2O_6S$ requires 365.1

(S)-2-[Allyl-(4-nitrobenzenesulfonyl)-amino]-3-phenylpropionic acid methyl ester (3.22) Lit[1]

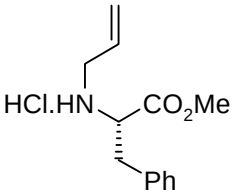


Sulfonamide **3.21** (4.50g, 12.4 mmol) was reacted with allyl bromide using General Procedure O1 to afford an orange oil, 4.68g, 93%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.22 (2H, d J=8.9Hz, Ar-**H** (4-NO_2 Ph)), 7.76 (2H, d J=8.9Hz, Ar-**H** (4-NO_2 Ph)), 7.30-7.34 (3H, m, Ar-**H** (Phe)), 7.23 (2H, dd J=1.9Hz, J=7.2Hz, Ar-**H** (Phe)), 5.69 (1H, dddd J=6.5Hz, J=6.5Hz, J=10.2Hz, J=15.1Hz, $NCH_2C\mathbf{H}CH_2$), 5.15-5.27 (2H, m, $NCH_2CHC\mathbf{H_2}$), 4.94 (1H, dd J=6.5Hz, J=8.8Hz, C**H**CO_2CH_3), 4.01 (1H, dd J=6.5Hz, J=16.3Hz, $NC\mathbf{H_2}CHCH_2$), 3.89 (1H, dd J=6.5Hz, J=16.3Hz, $NC\mathbf{H_2}CHCH_2$), 3.64 (3H, s, $CO_2C\mathbf{H_3}$), 3.42 (1H, dd J=6.5Hz, J=14.4Hz, CHC$\mathbf{H_2}$Ph), 3.04 (1H, dd J=8.8Hz, J=14.4Hz, CHC$\mathbf{H_2}$Ph)

LRMS (ES) 405.1 (MH^+). $C_{19}H_{20}N_2O_6S$ requires 405.1

(S)-2-Allylamino-3-phenylpropionic acid methyl ester (3.23) Lit[1]



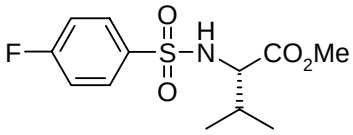
Methyl ester **3.22** (4.60g, 11.4 mmol) was dissolved in anhydrous DMF (30 mL). To this potassium carbonate (3 equiv) and thiophenol (1.2 equiv) were added. This was stirred at rt for one h. Diethyl ether (100 mL) and water (75 mL) were added. This was partitioned and the aqueous phase extracted twice more with diethyl ether. The

combined organic extracts were washed three times with 50% $NaHCO_{3(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether. To the combined product fractions 2M hydrogen chloride in diethyl ether (40 mL) was added. This was concentrated *in vacuo* to afford a pale yellow solid, 1.89g, 65%. R_f = 0.22 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (300 MHz in $CDCl_3$) 7.16-7.30 (5H, m, Ar-**H)**, 5.80 (1H, tdd J=6.0Hz, J=6.0Hz, J=10.2Hz, J=16.3Hz, $NHCH_2C\mathbf{H}CH_2$), 5.04-5.14 (2H, m, $NHCH_2CHC\mathbf{H_2}$), 3.63 (3H, s, $CO_2C\mathbf{H_3}$), 3.55 (1H, dd J=6.8Hz, J=6.8Hz, $C\mathbf{H}CO_2CH_3$), 3.26 (1H, dd J=6.8Hz, J=14.0Hz, $CHC\mathbf{H_2}Ph$), 3.10 (1H, dd J=6.8Hz, J=14.0Hz, $CHC\mathbf{H_2}Ph$), 2.95 (2H, d J=6.0Hz, $NC\mathbf{H_2}CHCH_2$)

LRMS (ES) 220.1 (MH^+). $C_{13}H_{17}NO_2$ requires 220.1

(S)-2-(4-Fluorobenzenesulfonylamino)-3-methylbutyric acid methyl ester (3.24) Lit[2]

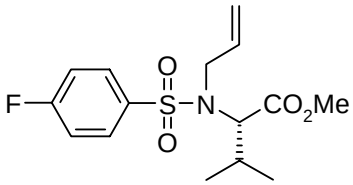


Val-OMe (2.00g, 11.9 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M2 to afford a white solid, 3.18g, 92%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.82-7.86 (2H, m, Ar-**H**), 7.13-7.19 (2H, m, Ar-**H**), 5.14 (1H, d J=10.1Hz, N**H**), 3.74 (1H, dd J=5.1Hz, J=10.1Hz, $C\mathbf{H}CO_2CH_3$), 3.48 (3H, s, $CO_2C\mathbf{H_3}$), 2.00-2.09 (1H, m, $CHC\mathbf{H}(CH_3)_3$), 0.95 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_3$), 0.87 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_3$)

LRMS (ES) 290.1 (MH^+). $C_{12}H_{16}FNO_4S$ requires 290.1

(S)-2-[Allyl-(4-fluorobenzenesulfonyl)amino]-3-methylbutyric acid methyl ester (3.25)

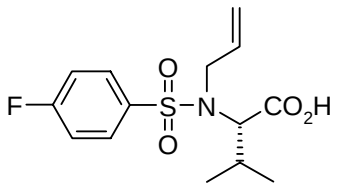


Sulfonamide **3.24** (1.00g, 3.46 mmol) was allylated using General Procedure O2 to afford a yellow oil, 1.01g, 89%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.81-7.85 (2H, m, Ar-**H**), 7.12-7.17 (2H, m, Ar-**H**), 5.80 (1H, dddd J=5.3Hz, J=7.7Hz, J=10.1Hz, J=17.6Hz, $NCH_2C\mathbf{H}CH_2$), 5.05-5.20 (2H, m, $NCH_2CHC\mathbf{H_2}$), 4.11 (1H, d J=10.6Hz, $C\mathbf{H}CO_2CH_3$), 4.03 (1H, dd J=7.7Hz, J=16.4Hz, $NC\mathbf{H_2}CHCH_2$), 3.88 (1H, dd J=5.3Hz, J=16.4Hz, $NC\mathbf{H_2}CHCH_2$), 3.44 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.09-2.17 (1H, m, $CHC\mathbf{H}(CH_3)_3$), 1.01 (3H, d J=6.6Hz, $CHCH(C\mathbf{H_3})_3$), 0.90 (3H, d J=6.6Hz, $CHCH(C\mathbf{H_3})_3$)

LRMS (ES) 330.1 (MH^+). $C_{15}H_{20}FNO_4S$ requires 330.1

(S)-2-[Allyl-(4-fluorobenzenesulfonyl)amino]-3-methylbutyric acid (3.26)

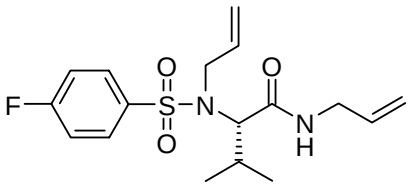


Methyl ester **3.25** (1.01g, 3.07 mmol) was hydrolysed using General procedure C1 to afford a white solid, 0.919g, 95%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.83-7.86 (2H, m, Ar-**H**), 7.11-7.16 (2H, m, Ar-**H**), 5.81 (1H, dddd J=5.7Hz, J=7.3Hz, J=10.1Hz, J=17.3Hz, $NCH_2C\mathbf{H}CH_2$), 5.07-5.21 (2H, m, $NCH_2CHC\mathbf{H_2}$), 4.13 (1H, d J=10.4Hz, $C\mathbf{H}CO_2CH_3$), 3.97 (1H, dd J=7.3Hz, J=16.4Hz, $NC\mathbf{H_2}CHCH_2$), 3.91 (1H, dd J=5.7Hz, J=16.4Hz, $NC\mathbf{H_2}CHCH_2$), 2.08-2.18 (1H, m, $CHC\mathbf{H}(CH_3)_3$), 0.98 (3H, d J=6.6Hz, $CHCH(C\mathbf{H_3})_3$), 0.98 (3H, d J=6.6 Hz, $CHCH(C\mathbf{H_3})_3$)

LRMS (ES) 316.1 (MH^+). $C_{14}H_{18}FNO_4S$ requires 316.1

(S)-N-Allyl-2-[allyl-(4-fluoro-benzenesulfonyl)amino]-3-methylbutyramide (3.28)

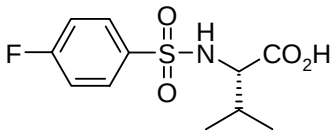


Carboxylic acid **3.26** (0.184g, 0.583 mmol) was reacted with allyl amine using General Procedure A3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.151g, 73%. R_f = 0.38 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.82-7.86 (2H, m, Ar-**H**), 7.15-7.19 (2H, m, Ar-**H**), 6.24 (1H, bs, N**H**), 5.74-5.83 (2H, m, $SO_2NCH_2C\mathbf{H}CH_2$ and $C(O)NHCH_2C\mathbf{H}CH_2$), 5.08-5.24 (4H, m, $SO_2NCH_2CHC\mathbf{H_2}$ and $C(O)NHCH_2CHC\mathbf{H_2}$), 4.10-4.13 (1H, m, $C(O)NHC\mathbf{H_2}CHCH_2$), 3.87-3.93 (1H, m, $C(O)NHC\mathbf{H_2}CHCH_2$), 3.80-3.85 (2H, m, $SO_2NC\mathbf{H_2}CHCH_2$), 3.69 (1H, d J=10.9Hz, $C\mathbf{H}CO_2CH_3$), 3.49 (3H, s, $CO_2C\mathbf{H_3}$), 2.19-2.28 (1H, m, $CHC\mathbf{H}(CH_3)_3$), 0.89 (3H, d J=6.4Hz, $CHCH(C\mathbf{H_3})_3$), 0.59 (3H, d J=6.4Hz, $CHCH(C\mathbf{H_3})_3$)

HRMS (ES) 355.1501 (MH^+). $C_{17}H_{23}FN_2O_3S$ requires 355.1491

(S)-2-(4-Fluorobenzenesulfonylamino)-3-methylbutyric acid (3.30) Lit[2]

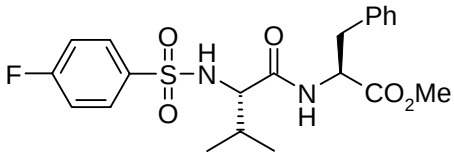


Methyl ester **3.24** (1.62g, 5.60 mmol) was hydrolysed using General Procedure C2 to afford a white solid, 1.54g, 100%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.15 (1H, bs, CO_2**H**), 7.83-7.86 (2H, m, Ar-**H**), 7.13-7.18 (2H, m, Ar-**H**), 5.25 (1H, d J=9.9Hz, N**H**), 3.77-3.80 (1H, m, C**H**CO_2CH_3), 2.08-2.15 (1H, m, CHC**H**$(CH_3)_3$), 0.97 (3H, d J=6.8Hz, CHCH(C**H$_3$**$)_3$), 0.88 (3H, d J=6.8Hz, CHCH(C**H$_3$**$)_3$)

LRMS (ES) 276.1 (MH^+). $C_{11}H_{14}FNO_4S$ requires 276.1

(S)-2-[(S)-2-(4-Fluorobenzenesulfonylamino)-3-methylbutyrylamino]-3-phenylpropionic acid methyl ester (3.31)



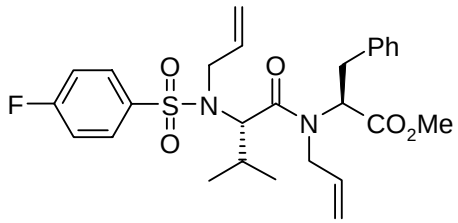
Carboxylic acid **3.30** (0.500g, 1.82 mmol) was reacted with Phe-OMe using General Procedure A3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.704g, 86%. R_f = 0.27 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.86-7.90 (2H, m, Ar-**H** (4-F-Ph)), 7.26-7.30 (3H, m, Ar-**H** (Phe)), 7.14-7.19 (2H, m, Ar-**H** (4-F-Ph)), 6.95 (2H, dd J=1.9Hz, J=7.3Hz, Ar-**H** (Phe)), 6.10 (1H, d J=7.6Hz, N**H** Phe), 5.40 (1H, d J=8.6Hz, N**H** Val), 4.68 (1H, ddd J=5.9Hz, J=5.9Hz, J=7.6Hz, C**H**CH_2Ph), 3.69 (3H, s, CO_2C**H$_3$**), 3.53 (1H, dd J=4.8Hz, J=8.6Hz, C**H**$CH(CH_3)_3$), 2.98 (1H, dd J=5.9Hz, J=14.0Hz, CHC**H$_2$**Ph), 2.92 (1H, dd J=5.9Hz, J=14.0Hz, CHC**H$_2$**Ph), 1.96-2.05 (1H, m, CHC**H**$(CH_3)_3$), 0.88 (3H, d J=6.8Hz, CHCH(C**H$_3$**$)_3$), 0.78 (3H, d J=6.8Hz, CHCH(C**H$_3$**$)_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.3, 169.7, 135.3, 130.1, 130.0, 129.0, 128.7, 127.3, 116.3, 116.0, 61.6, 53.3, 52.4, 37.7, 31.7, 19.0, 16.9

LRMS (ES) 437.1 (MH^+). $C_{21}H_{25}FN_2O_5S$ requires 437.1

(S)-2-(Allyl-{(S)-2-[allyl-(4-fluorobenzenesulfonyl)amino]-3-methylbutyryl}amino)-3-phenylpropionic acid methyl ester (3.32)



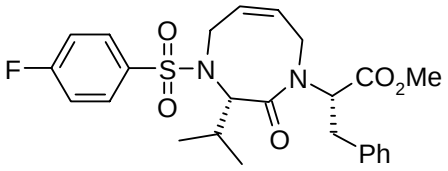
Dipeptide **3.31** (0.500g, 1.09 mmol) was allylated using General Procedure O3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.300g, 51%. R_f = 0.37 (1/7 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.80-7.86 (2H, m, Ar-**H** (4-F-Ph)), 7.24-7.39 (5H, m, Ar-**H** (Phe) and Ar-**H** (4-F-Ph)), 7.11-7.17 (2H, m, Ar-**H** (Phe)), 5.48-5.68 (2H, m, $SO_2NCH_2C\mathbf{H}CH_2$ and $C(O)NCH_2C\mathbf{H}CH_2$), 5.06-5.29 (4H, m, $SO_2NCH_2CHC\mathbf{H_2}$ and $C(O)NCH_2CHC\mathbf{H_2}$), 4.93 (1H, dd J=1.7Hz, J=10.1Hz, $SO_2NC\mathbf{H_2}CHCH_2$), 4.60 (1H, dd J=1.9, J=10.4Hz, $SO_2NC\mathbf{H_2}CHCH_2$), 4.52-4.57 (1H, m, $C\mathbf{H}CH_2Ph$), 4.00-4.15 (2H, m, $C(O)NC\mathbf{H_2}CHCH_2$ and $C\mathbf{H}CH(CH_3)_3$), 3.94 (1H, dd J=3.5Hz, J=16.0Hz, $C(O)NC\mathbf{H_2}CHCH_2$), 3.68 (3H, s, $CO_2C\mathbf{H_3}$), 3.55 (1H, dd J=3.9Hz, J=13.5Hz, $CHC\mathbf{H_2}Ph$), 3.09 (1H, dd J=9.4Hz, J=13.5Hz, $CHC\mathbf{H_2}Ph$), 2.34-2.39 (1H, m, $CHC\mathbf{H}(CH_3)_3$), 0.92 (3H, d J=6.3Hz, $CHCH(C\mathbf{H_3})_3$), 0.89 (3H, d J=6.3Hz, $CHCH(C\mathbf{H_3})_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 170.6, 170.4, 166.5, 163.1, 137.7, 136.6, 136.6, 134.6, 133.6, 130.4, 130.3, 129.1, 128.4, 126.6, 119.0, 117.3, 116.0, 115.7, 60.7, 60.7, 52.0, 51.4, 46.8, 34.9, 28.8, 19.5, 19.4

HRMS (ES) 517.2179 (MH^+). $C_{27}H_{33}FN_2O_5S$ requires 517.2172

(S)-2-[(Z)-(S)-4-(4-Fluorobenzenesulfonyl)-3-isopropyl-2-oxo-3,4,5,8-tetrahydro-2H-[1,4]-diazocin-1-yl]-3-phenylpropionic acid methyl ester (3.33)



Diene **3.32** (0.100g, 0.189 mmol) was ring closed using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.0568g, 60%. R_f = 0.16 (1/3 (EtOAc / (50/70) Pet ether)).

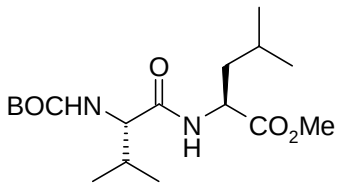
^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.80-7.85 (2H, m, Ar-**H** (4-F-Ph)), 7.27 (2H, dd J=7.3Hz, J=7.3Hz, Ar-**H** (Phe)), 7.12-7.22 (5H, m, Ar-**H** (4-F-Ph) and Ar-**H** (Phe)), 5.80 (1H, ddd J=4.8Hz, J=4.8Hz, J=9.8Hz, $SO_2NCH_2C\mathbf{H}CHCH_2N$), 5.41-5.47 (1H, m, $SO_2NCH_2CHC\mathbf{H}CH_2N$), 4.32-4.37 (2H, m, $CHC\mathbf{H_2}Ph$ and $C\mathbf{H}CH(CH_3)_3$), 4.22 (1H, dd J=4.8Hz, J=17.3Hz, $SO_2NC\mathbf{H_2}CHCHCH_2N$), 3.99 (1H, dd J=5.9Hz, J=16.2Hz, $SO_2NCH_2CHCHC\mathbf{H_2}N$), 3.74 (1H, dd J=4.8Hz, J=17.3Hz, $SO_2NC\mathbf{H_2}CHCHCH_2N$), 3.66 (3H, s, $CO_2C\mathbf{H_3}$), 3.33 (1H, dd J=5.2Hz, J=14.2Hz, $CHC\mathbf{H_2}Ph$), 3.26 (1H, dd J=8.4Hz, J=16.2Hz, $SO_2NCH_2CHCHC\mathbf{H_2}N$), 3.09 (1H, dd

J=10.2Hz, J=14.2Hz, CHC**H**$_2$Ph), 2.09-2.18 (1H, m, CHC**H**$(CH_3)_3$), 0.82 (3H, d J=6.6Hz, CHCH(C**H**$_3)_3$), 0.79 (3H, d J=6.6Hz, CHCH(C**H**$_3)_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 170.5, 168.8, 166.6, 163.2, 137.4, 136.3, 132.5, 130.0, 129.9, 129.2, 128.5, 127.1, 126.6, 116.2, 115.9, 67.3, 63.3, 52.0, 44.2 43.3, 35.0, 30.1, 20.1, 17.0

HRMS (ES) 489.1862 (MH^+). $C_{25}H_{29}FN_2O_5S$ requires 489.1859

(S)-2-((S)-2-tert-Butoxycarbonylamino-3-methylbutyrylamino)-4-methylpentanoic acid methyl ester (3.35) Lit[3]



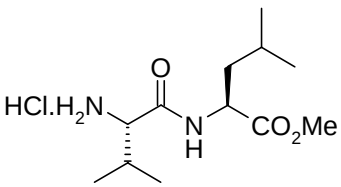
N-BOC-Val-H (3.67g, 17.0 mmol) was reacted with Leu-OMe using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 5.44g, 93%. R_f = 0.39 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 6.59 (1H, d J=7.0 Hz, N**H** Val), 5.17 (1H, d J=8.1 Hz, N**H** Leu), 4.53-4.62 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.92 (1H, dd J=6.6Hz, J=7.0Hz, C**H**$CH(CH_3)_2$), 3.68 (1H, s, CO_2C**H**$_3$), 1.91-2.20 (1H, m, CHC**H**$(CH_3)_2$), 1.47-1.70 (3H, m, CHC**H**$_2CH(CH_3)_2$ and $CHCH_2C$**H**$(CH_3)_2$), 1.40 (1H, s, C(C**H**$_3)_3$), 0.86-0.94 (12H, m, $CHCH_2CH(C$**H**$_3)_2$ and CHCH(C**H**$_3)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 173.1, 171.4, 163.3, 59.9, 52.2, 50.6, 41.4, 31.8, 30.8, 28.2, 24.7, 22.8, 19.2, 17.8

LRMS (ES) 345.2 (MH^+). $C_{17}H_{32}N_2O_5$ requires 345.2

(S)-2-((S)-2-Amino-3-methylbutyrylamino)-4-methylpentanoic acid methyl ester hydrogen chloride salt (3.36) Lit[3]

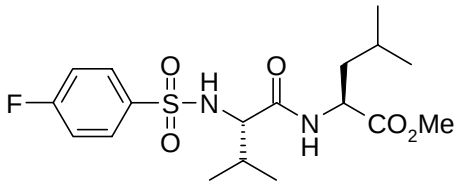


N-BOC protected compound **3.35** (0.980g, 2.85 mmol) was reacted using General Procedure I3 to afford a white solid, 0.799g, 100%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.26 (3H, bs, NH_2 and N**H** Leu), 4.35-4.40 (1H, m, C**H**$CH_2CH(CH_3)_2$), 4.11-4.29 (1H, m, C**H**$CH(CH_3)_2$), 3.72 (3H, s, CO_2C**H**$_3$), 2.15-2.24 (1H, m, CHC**H**$(CH_3)_2$), 1.47-1.62 (3H, m, CHC**H**$_2CH(CH_3)_2$)and $CHCH_2C$**H**$(CH_3)_2$), 0.92-0.97 (12H, m $CHCH_2CH(C$**H**$_3)_2$ and CHCH(C**H**$_3)_2$)

LRMS (ES) 245.2 (MH^+). $C_{12}H_{24}N_2O_3$ requires 245.2

(S)-2-[(S)-2-(4-Fluorobenzenesulfonylamino)-3-methylbutyrylamino]-4-methylpentanoic acid methyl ester (3.37) Lit[2]



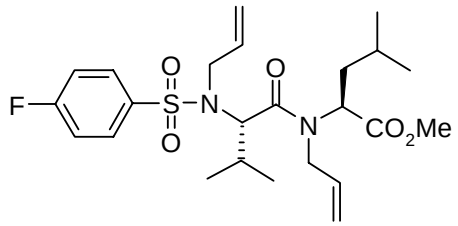
Amine **3.36** (0.800g, 2.85 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.270g, 24%. R_f = 0.26 (1/3 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.83-7.89 (2H, m, Ar-**H**), 7.10-7.16 (2H, m, Ar-**H**), 6.16 (1H, d J=8.1Hz, N**H** Val), 5.63 (1H, d J=8.8Hz, N**H** Leu), 4.35-4.44 (1H, m, C**H**$CH_2CH(CH_3)_2$), 4.11 (1H, dd J=7.1Hz, J=8.1Hz, C**H**$CH(CH_3)_2$), 3.69 (3H, s, $CO_2C\mathbf{H_3}$), 1.99-2.08 (1H, m, $CHCH(C\mathbf{H_3})_2$), 1.41-1.54 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 1.21-1.37 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 0.94 (3H, d J=6.8 Hz, $CHCH(C\mathbf{H_3})_2$), 0.84 (3H, d J=6.8 Hz, $CHCH(C\mathbf{H_3})_2$), 0.84 (3H, d, J=6.5 Hz, $CHCH_2CH(C\mathbf{H_3})_2$), 0.80 (3H, d J=6.5 Hz, $CHCH_2CH(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.8, 170.1, 165.9, 163.9, 135.8, 130.0, 129.9, 116.1, 115.9, 61.4, 52.2, 50.6, 41.1, 31.8, 24.4, 22.4, 21.5, 19.0, 17.0

LRMS (ES) 403.2 (MH^+). $C_{18}H_{27}FN_2O_5S$ requires 403.2

(S)-2-(Allyl-{(S)-2-[allyl-(4-fluorobenzenesulfonyl)amino]-3-methylbutyryl}amino)-4-methylpentanoic acid methyl ester (3.38)



Dipeptide **3.37** (0.671g, 1.67 mmol) was allylated using General Procedure O3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil 0.301g, 37%. R_f = 0.52 (1/4 (EtOAc / (50/70) Pet ether)).

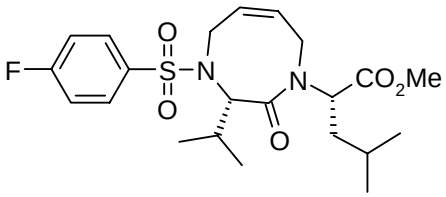
^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.78-7.88 (2H, m, Ar-**H**), 7.11-7.18 (2H, m, Ar-**H**), 5.89 (1H, dddd, J=5.6Hz, J=7.3Hz, J=10.1Hz, J=15.8Hz, $CHNCH_2C\mathbf{H}CH_2$), 5.59-5.68 (1H, m, $SO_2NCH_2C\mathbf{H}CH_2$), 4.85-5.41 (2H, m, $SO_2NCH_2C\mathbf{H}CH_2$ and $CHNCH_2C\mathbf{H}CH_2$, 4.67 (1H, dd, J=6.1Hz, J=8.5Hz, C**H**$CH_2CH(CH_3)_2$), 4.62 (1H, d J=10.5Hz, C**H**$CH(CH_3)_2$), 4.40 (1H, dd, J=7.4Hz, J=16.6Hz, $CHNC\mathbf{H_2}CHCH_2$), 4.18 (1H, dd, J=8.0Hz, J=16.4Hz, $SO_2NC\mathbf{H_2}CHCH_2$), 3.96 (2H, m, $CHNC\mathbf{H_2}CHCH_2$ and $SO_2NC\mathbf{H_2}CHCH_2$) 3.66 (3H, s, $CO_2C\mathbf{H_3}$),

2.23-2.39 (1H, m, CHC**H**$(CH_3)_2$), 1.87-1.96 (1H, m, $CHCH_2C$**H**$(CH_3)_2$), 1.48-1.67 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 0.87-0.93 (12H, m, $CHCH_2CH(C$**H$_3$**$)_2$ and $CHCH(C$**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 171.8, 171.1, 166.6, 163.3, 134.8, 134.4, 130.5, 130.4, 118.7, 117.4, 116.1, 115.8, 60.6, 55.9, 52.0, 49.6, 47.0, 37.6, 29.1, 24.9, 22.8, 22.0, 19.7, 19.2

HRMS (ES) 483.2320 (MH^+). $C_{24}H_{35F}N_2O_5S$ requires 483.2329

(S)-2-[(Z)-(S)-4-(4-Fluorobenzenesulfonyl)-3-isopropyl-2-oxo-3,4,5,8-tetrahydro-2H-[1,4]diazocin-1-yl]-4-methylpentanoic acid methyl ester (3.39)



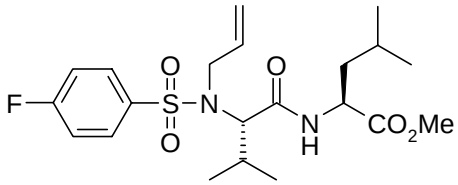
Diene **3.38** (0.100g, 0.189 mmol) was ring closed using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.0568g, 60%. R_f = 0.16 (1/3 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.80-7.88 (2H, m, Ar-**H**), 7.11-7.17 (2H, m, Ar-**H**), 5.88 (1H, td, J=4.3Hz, J=7.3z, J=10.1z,SO_2CH_2CHC**H**CH_2), 5.75 (1H, m, SO_2CH_2C**H**$CHCH_2$), 4.83 (1H, dd J=5.8Hz, J=8.5Hz, C**H**$CH_2CH(CH_3)_2$), 4.31-4.40 (2H, m, C**H**$CH(CH_3)_2$ and SO_2CH_2CHCHC**H$_2$**) 4.26 (1H, dd, J=7.0Hz, J=16.0Hz, SO_2C**H$_2$**$CHCHCH_2$), 3.82 (1H, dd J=4.3Hz, J=16.0Hz, SO_2C**H$_2$**$CHCHCH_2$), 3.68 (1H, dd J=7.9z, J=15.5Hz, SO_2CH_2CHCHC**H$_2$**), 3.65 (3H, s, $CHCO_2C$**H$_3$**), 2.17-2.29 (1H, m, CHC**H**$(CH_3)_2$), 1.71-1.81 (1H, m, $CHCH_2C$**H**$(CH_3)_2$), 1.43-1.56 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 0.92 (3H, d J=6.7Hz, $CHCH_2CH(C$**H$_3$**$)_2$), 0.89 (6H, m, $CHCH_2CH(C$**H$_3$**$)_2$and $CHCH(C$**H$_3$**$)_2$), 0.79 (3H, d J=6.7Hz, $CHCH(C$**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 171.9, 169.3, 166.5, 163.2, 136.1, 131.5, 130.1, 130.0, 128.7, 116.1, 115.9, 67.9, 57.1, 51.9, 43.2, 41.6,38.2, 29.7, 24.7, 22.8, 21.8, 20.5, 18.2

HRMS (ES) 455.2016 (MH^+). $C_{22}H_{31}FN_2O_5S$ requires 455.2016

(S)-2-{(S)-2-[Allyl-(4-fluorobenzenesulfonyl)amino]-3-methyl-butyrylamino}-4-methyl-pentanoic acid methyl ester (3.41)



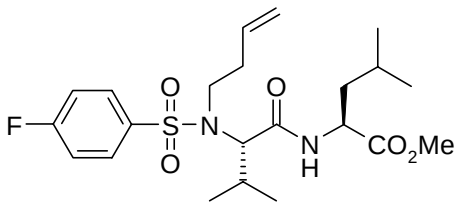
Sulfonamide **3.37** (1.00g, 2.48 mmol) was dissolved in anhydrous DMF (20 mL) under an atmosphere of argon. To this potassium carbonate (2 equiv) and allyl bromide (4 equiv) were added. This was stirred at rt for eighteen h before being diluted with EtOAc and partitioned with 1M $HCl_{(aq)}$. The aqueous phase was extracted twice

more with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a colourless oil, 0.857g, 78%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.78-7.91 (2H, m, Ar-**H**), 7.12-7.17 (2H, m, Ar-**H**), 6.51 (1H, d J=7.5Hz, N**H**), 5.74 (1H, tdd J=6.7Hz, J=6.7Hz, J=10.1Hz, J=16.8Hz, $NHCH_2C\mathbf{H}CH_2$), 5.03-5.23 (2H, m, $NHCH_2CHC\mathbf{H_2}$), 4.41-4.50 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 4.06 (1H, dd J=6.7Hz, J=16.1Hz, $NHC\mathbf{H_2}CHCH_2$), 3.89 (1H, dd, J=6.7Hz, J=16.1Hz, $NHC\mathbf{H_2}CHCH_2$), 3.76 (1H, d J=10.9Hz, $C\mathbf{H}CH(CH_3)_2$), 3.71 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.14-2.25 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.56-1.68 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 1.46-1.54 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 0.92 (3H, d J=6.0 Hz, $CHCH_2CH(C\mathbf{H_3})_2$), 0.91 (3H, d J=6.0Hz, $CHCH_2CH(C\mathbf{H_3})_2$), 0.87 (3H, d J=6.7Hz, $CHCH(C\mathbf{H_3})_2$), 0.58 (3H, d J=6.7Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 443.2108 (MH^+). $C_{21}H_{31}FN_2O_5S$ requires 443.2016

(S)-2-{(S)-2-[But-3-enyl-(4-fluorobenzenesulfonyl)amino]-3-methylbutyrylamino}-4-methyl pentanoic acid methyl ester (3.42)



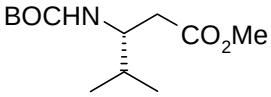
Sulfonamide **3.37** (1.00g, 2.48 mmol) was dissolved in anhydrous DMF (20 mL) under an atmosphere of argon. To this potassium carbonate (2 equiv) and 4-bromo-1-butene (4 equiv) were added. This was stirred at rt for eighteen h before being diluted with EtOAc and partitioned with 1M $HCl_{(aq)}$. The aqueous phase was extracted twice more with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a colourless oil, 0.839g, 74%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.82-7.86 (2H, m, Ar-**H**), 7.13-7.19 (2H, m, Ar-**H)**, 6.69 (1H, d J=7.1Hz, N**H** Leu), 5.64-5.73 (1H, m, $CH_2CH_2C\mathbf{H}CH_2$), 4.96-5.06 (2H, m, $CH_2CH_2CHC\mathbf{H_2}$), 4.46-4.51 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 3.69-3.73 (4H, m, $CO_2C\mathbf{H_3}$ and $C\mathbf{H}CH(CH_3)_2$), 3.40-3.48 (1H, m, $C\mathbf{H_2}CH_2CHCH_2$), 3.14-3.21 (1H, m, $C\mathbf{H_2}CH_2CHCH_2$), 2.29-2.45 (2H, m, $CH_2C\mathbf{H_2}CHCH_2$), 2.14-2.22 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 1.52-1.73 (3H, m, $CHC\mathbf{H_2}CH(CH_3)_2$ and $CHCH_2C\mathbf{H}(CH_3)_2$), 0.92-0.96 (6H, m, $CHCH_2CH(C\mathbf{H_3})_2$), 0.88 (3H, d J=6.3Hz, $CHCH(C\mathbf{H_3})_2$), 0.49 (3H, d J=6.3Hz, $CHCH(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 173.0, 169.8, 134.6, 129.8, 129.7, 117.0, 116.4, 116.1, 65.7, 52.2, 50.8, 44.5, 40.8, 34.5, 26.8, 24.6, 22.7, 21.6, 19.7, 18.9

HRMS (ES) 457.2183 (MH^+). $C_{22}H_{33}FN_2O_5S$ requires 457.2172

(R)-3-tert-Butoxycarbonylamino-4-methylpentanoic acid methyl ester (3.50) Lit[4]

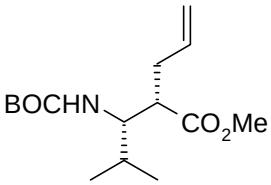


N-BOC-Val-H (5.00g, 43.0 mmol) was homologated using General Procedure P. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 6.86g, 65%. R_f = 0.47 (1/9 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 4.86 (1H, d J=8.7Hz, N**H**), 3.74-3.79 (1H, m, C**H**CH$(CH_3)_2$), 3.64 (3H, s, $CO_2C\mathbf{H_3}$), 2.53 (1H, dd J=5.0Hz, J=15.2Hz, C$\mathbf{H_2}CO_2CH_3$), 2.47 (1H, dd J=6.9Hz, J=15.2Hz, C$\mathbf{H_2}CO_2CH_3$), 1.77-1.85 (1H, m, CHC**H**$(CH_3)_2$) , 1.40 (9H, s, C(C$\mathbf{H_3}$)$_3$), 0.88 (6H, d J=6.8Hz, CHCH(C$\mathbf{H_3}$)$_2$)

LRMS (ES) 246.2 (MH^+). $C_{12}H_{23}NO_4$ requires 246.2

(S)-2-((S)-1-tert-Butoxycarbonylamino-2-methylpropyl)pent-4-enoic acid methyl ester (3.51)

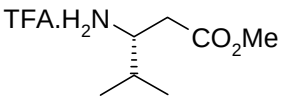


β-amino acid **3.50** (0.575g, 2.34 mmol) was allylated using General Procedure Q. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 0.107g, 16%. R_f = 0.22 (1/9 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 5.76 (1H, dddd J=7.1Hz, J=7.1Hz, J=10.1Hz, J=17.1Hz, $CH_2C\mathbf{H}CH_2$), 5.30 (1H, d J=10.3Hz, N**H**), 5.01-5.11 (2H, m, $CH_2CHC\mathbf{H_2}$), 3.64 (3H, s, $CO_2C\mathbf{H_3}$), 3.47 (1H, ddd J=4.0Hz, J=8.8Hz, J=10.3Hz, C**H**CH$(CH_3)_2$), 2.73 (1H, ddd J=4.0Hz, J=6.5Hz, J=8.5Hz, C**H**CO_2CH_3), 2.34-2.40 (1H, m, C$\mathbf{H_2}CHCH_2$), 2.24-2.30 (1H, m, C$\mathbf{H_2}CHCH_2$), 1.56 (1H, m, CHC**H**$(CH_3)_2$), 1.41 (9H, s, C(C$\mathbf{H_3}$)$_3$), 0.94 (3H, d J=6.7Hz, CHCH(C$\mathbf{H_3}$)$_2$), 0.92 (3H, d J=6.7Hz, CHCH(C$\mathbf{H_3}$)$_2$)

LRMS (ES) 286.2 (MH^+). $C_{15}H_{27}NO_4$ requires 286.2

(R)-3-Amino-4-methylpentanoic acid methyl ester trifluoroacetic acid salt (3.53) Lit[2]

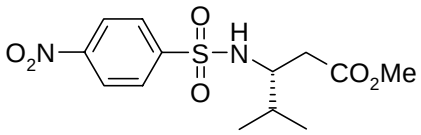


N-BOC protected compound **3.50** (0.200g, 0.815 mmol) was reacted using General Procedure I2 to afford a yellow oil, 0.211g, 100%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.51 (2H, bs, N$\mathbf{H_2}$), 3.75 (3H, s, $CO_2C\mathbf{H_3}$), 3.39-3.51 (1H, m, C**H**CH$(CH_3)_2$), 2.76 (1H, dd J=3.7Hz, J=17.9Hz, C$\mathbf{H_2}CO_2CH_3$), 2.70 (1H, dd J=9.3Hz, J=17.9Hz, C$\mathbf{H_2}CO_2CH_3$), 1.97-2.10 (1H, m, CHC**H**$(CH_3)_2$), 1.05 (3H, d J=6.9Hz, CHCH(C$\mathbf{H_3}$)$_2$), 1.01 (3H, d J=6.9Hz, CHCH(C$\mathbf{H_3}$)$_2$)

LRMS (ES) 146.1 (MH^+). $C_7H_{15}NO_2$ requires 146.1

(R)-4-Methyl-3-(4-nitrobenzenesulfonylamino)pentanoic acid methyl ester (3.54)



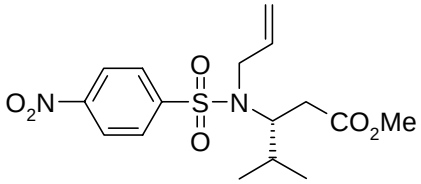
Amine **3.53** (0.210g, 0.815 mmol) was reacted with 4-nitrobenzene sulfonyl chloride using General Procedure M1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow oil, 0.0806g, 30%. R_f = 0.28 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (300 MHz in $CDCl_3$) 8.35 (2H, d J=8.8Hz, Ar-**H**), 8.06 (2H, d J=8.8Hz, Ar-**H**), 5.44 (1H, d J=9.0Hz, N**H**), 3.59 (3H, s, $CO_2C\mathbf{H_3}$), 3.40-3.44 (1H, m, C**H**$CH(CH_3)_3$), 2.48 (1H, dd J=5.2Hz, J=15.8Hz, $C\mathbf{H_2}CO_2CH_3$), 2.37 (1H, dd J=5.2Hz, J=15.8Hz, $C\mathbf{H_2}CO_2CH_3$), 1.78-1.89 (1H, m, CHC**H**$(CH_3)_3$), 0.84 (3H, d J=6.7Hz, $CHCH(C\mathbf{H_3})_3$), 0.83 (3H, d J=6.7 Hz, $CHCH(C\mathbf{H_3})_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 171.8, 128.2, 124.2, 56.8, 51.9, 36.2, 31.9, 18.8, 18.6

LRMS (ES) 331.1 (MH^+). $C_{13}H_{18}N_2O_6S$ requires 331.1

(R)-3-[Allyl-(4-nitrobenzenesulfonyl)amino]-4-methylpentanoic acid methyl ester (3.55)



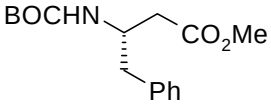
Sulfonamide **3.54** (0.136g, 0.412 mmol) was allylated using General Procedure O2 to afford a yellow oil, 0.152g, 100%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.31 (2H, d J=8.9Hz, Ar-**H**), 8.04 (2H, d J=8.9Hz, Ar-**H**), 5.71-5.82 (1H, m, $CH_2C\mathbf{H}CH_2$), 5.11-5.26 (2H, m, $CH_2CHC\mathbf{H_2}$), 4.05 (1H, ddd J=6.4Hz, J=6.4Hz, J=10.0Hz, C**H**$CH(CH_3)_2$), 3.88 (1H, dd J=6.8Hz, J=16.1Hz, $C\mathbf{H_2}CHCH_2$), 3.81 (1H, dd J=7.1Hz, J=16.1Hz, $C\mathbf{H_2}CHCH_2$), 3.57 (3H, s, $CO_2C\mathbf{H_3}$), 2.58 (1H, dd, J=6.4Hz, J=15.8Hz, $C\mathbf{H_2}CO_2CH_3$), 2.37 (1H, dd J=6.4Hz, J=15.8Hz, $C\mathbf{H_2}CO_2CH_3$), 1.81-1.91 (1H, m, CHC**H**$(CH_3)_2$), 0.91 (3H, d J=7.1Hz, $CHCH(C\mathbf{H_3})_2$), 0.88 (3H, d J=7.1 Hz, $CHCH(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.5, 146.7, 134.4, 128.8, 124.0, 118.8, 62.3, 51.9, 47.8, 37.7, 32.1, 20.5, 19.5

HRMS (ES) 371.1291 (MH^+). $C_{16}H_{22}N_2O_6S$ requires 371.1277

(S)-3-tertButoxycarbonylamino-4-phenylbutyric acid methyl ester (3.57) Lit[5]

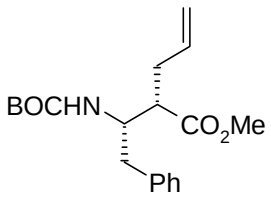


N-BOC-Phe-H (8.00g, 32.1 mmol) was homologated using General Procedure P. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow oil, 5.84g, 62%. R_f = 0.39 (1/5 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (300 MHz in $CDCl_3$) 7.21-7.33 (5H, m, Ar-**H**), 5.13 (1H, d J=6.2Hz, N**H**), 4.17-4.20 (1H, m, C**H**$CH_2CO_2CH_3$), 3.71 (3H, s, $CO_2C\mathbf{H_3}$), 2.97 (1H, dd J=5.9Hz, J=12.7Hz, CHC$\mathbf{H_2}$Ph), 2.84 (1H, dd J=7.6Hz, J=12.7Hz, CHC$\mathbf{H_2}$Ph), 2.50 (1H, dd J=5.3Hz, J=15.3Hz, CHC$\mathbf{H_2}CO_2CH_3$), 2.48 (1H, dd J=5.3Hz, J=15.3Hz CHC$\mathbf{H_2}CO_2CH_3$), 1.44 (9H, s, (C$\mathbf{H_3}$)$_3$)

LRMS (ES) 294.2 (MH^+). $C_{16}H_{23}NO_4$ requires 294.2

(S)-2-((S)-1-tert-Butoxycarbonylamino-2-phenylethyl)pent-4-enoic acid methyl ester (3.58)

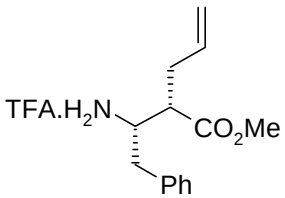


β-amino acid **3.57** (0.500g, 1.70 mmol) was allylated using General Procedure Q. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow oil, 0.148g, 26%. R_f = 0.47 (1/5 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.22-7.33 (5H, m, Ar-**H**), 5.70 (1H, dddd, 1H, J=7.0Hz, J=7.0Hz, J=10.1Hz, J=17.1Hz, $CH_2C\mathbf{H}CH_2$), 5.53 (1H, d J=9.7Hz, N**H**), 4.96-5.10 (2H, m, $CH_2CHC\mathbf{H_2}$), 4.02-4.12 (1H, m, C**H**CH_2Ph), 3.76 (3H, s, $CO_2C\mathbf{H_3}$), 2.86-2.92 (1H, m, C**H**CO_2CH_3), 2.60-2.70 (2H, m, CHC$\mathbf{H_2}$Ph), 2.42-2.48 (1H, m, C$\mathbf{H_2}CHCH_2$), 2.32 (1H, ddd J=6.8Hz, J=7.0Hz, J=13.7Hz, C$\mathbf{H_2}CHCH_2$), 1.43 (9H, s, (C$\mathbf{H_3}$)$_3$)

LRMS (ES) 334.2 (MH^+). $C_{19}H_{27}NO_4$ requires 334.2

(S)-2-((S)-1-Amino-2-phenylethyl)-pent-4-enoic acid methyl ester trifluoroacetic acid salt (3.59)

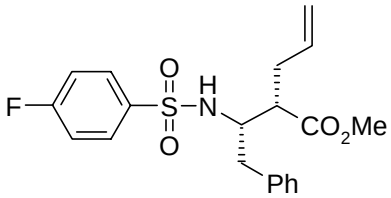


N-BOC protected compound **3.58** (0.148g, 0.444 mmol) was reacted using General Procedure I2 to afford a yellow oil, 0.154g, 100%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.58 (2H, bs, N**H$_2$**), 7.33-7.40 (3H, m, Ar-**H**), 7.16 (2H, m, Ar-**H**), 5.60 (1H, tdd J=7.0Hz, J=9.8Hz, J=16.9Hz, CH_2C**H**CH_2), 5.13-5.17 (2H, m, CH_2CHC**H$_2$**), 3.79 (3H, s, CO_2C**H$_3$**), 3.75-3.78 (1H, m, C**H**CH_2Ph), 3.08 (1H, dd J=7.2Hz, J=14.1Hz, CHC**H$_2$**Ph), 2.98 (1H, dd J=8.0Hz, J=14.1Hz, CHC**H$_2$**Ph), 2.81 (1H, tdd J=4.7Hz, J=7.0Hz, J=7.0Hz, C**H**CO_2CH_3), 2.55 (2H, dd J=7.0Hz J=7.0Hz, C**H$_2$**$CHCH_2$)

LRMS (ES) 234.2 (MH^+). $C_{14}H_{19}NO_2$ requires 324.1

(S)-2-[(S)-1-(4-Fluoro-benzenesulfonylamino)-2-phenylethyl]pent-4-enoic acid methyl ester (3.60)



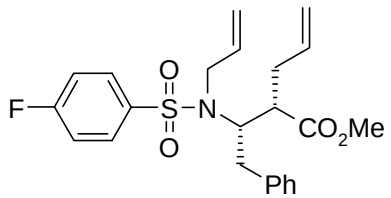
Amine **3.59** (0.154g, 0.444 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.174g, 100%. R_f = 0.32 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.77-7.79 (2H, m, Ar-**H** (4-F-Ph)), 7.20-7.24 (3H, m, Ar-**H** (Phe)), 7.11-7.16 (2H, m, Ar-**H** (4-F-Ph)), 7.02 (2H, dd J=1.0Hz, J=6.9Hz, Ar-**H** (Phe)), 5.67 (1H, d J=9.0Hz, N**H**), 5.51-5.59 (1H, m, CH_2C**H**CH_2), 4.91-5.01 (2H, m, CH_2CHC**H$_2$**), 3.68-3.73 (4H, m, C**H**CH_2Ph and CO_2C**H$_3$**), 2.77 (1H, dd J=5.8Hz, J=13.8Hz, CHC**H$_2$**Ph), 2.56-2.67 (2H, m, CHC**H$_2$**Ph and C**H**CO_2CH_3), 2.34 (1H, m, C**H$_2$**$CHCH_2$), 2.17 (1H, m, C**H$_2$**$CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 174.5, 134.1, 129.5, 129.4, 129.1, 128.6, 126.8, 117.5, 116.3, 116.0, 56.1, 51.8, 46.2, 40.9, 33.7

LRMS (ES) 392.2 (MH^+). $C_{20}H_{22}FNO_4S$ requires 392.1

(S)-2-{(S)-1-[Allyl-(4-fluorobenzenesulfonyl)amino]-2-phenylethyl}pent-4-enoic acid methyl ester (3.61)



Sulfonamide **3.60** (0.200g, 0.511 mmol) was allylated using General Procedure O2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.180g, 82%. R_f = 0.27 (1/7 (EtOAc / (50/70) Pet ether)).

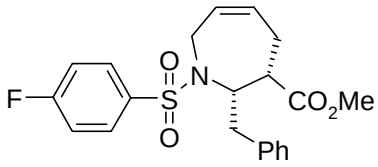
^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.42-7.48 (2H, m, Ar-**H** (4-F-Ph)), 7.25-7.35 (5H, m, Ar-**H** (Phe)), 6.97-7.03 (2H, m, Ar-**H** (4-F-Ph)), 5.76 (1H, dddd, 1H, J=2.2Hz, J=4.5Hz, J=9.8Hz, J=12.8Hz, NCH_2C**H**CH_2), 5.49-5.58 (1H, m, $CHCH_2C$**H**CH_2), 5.06-5.39 (4H, m, NCH_2CHC**H$_2$** and $CHCH_2CHC$**H$_2$**), 4.58-4.63 (1H, m, C**H**CH_2Ph),

3.74-3.87 (2H, m, NC**H**$_2$CHCH$_2$), 3.68 (3H, s, CO$_2$C**H**$_3$), 3.11 (1H, dd J=6.6Hz, J=13.9Hz, CHC**H**$_2$Ph), 3.04 (1H, dd J=7.6Hz, J=13.9Hz, CHC**H**$_2$Ph), 2.87-2.94 (1H, m, C**H**CO$_2$CH$_3$), 2.40-2.48 (2H, m, C**H**$_2$CHCH$_2$)

^{13}C NMR ppm (75 MHz in CDCl$_3$) 173.2, 130.5, 134.8, 134.2, 130.4, 129.4, 128.6, 126.7, 118.0, 117.5, 115.8, 115.5, 62.1, 51.7, 49.9, 37.5, 34.5

HRMS (ES) 432.1658 (MH$^+$). $C_{23}H_{26}FNO_4S$ requires 432.1645

(2S,3S)-2-Benzyl-1-(4-fluorobenzenesulfonyl)-2,3,4,7-tetrahydro-1H-azepine-3-carboxylic acid methyl ester (3.62)



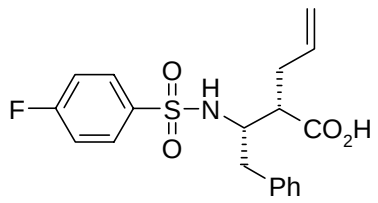
Diene **3.61** (0.174g, 0.403 mmol) was reacted with Grubbs second generation catalyst using General Procedure D1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.132g, 81%. R_f = 0.19 (1/5 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CDCl$_3$) 7.36-7.40 (2H, m, Ar-**H** (4-F-Ph)), 7.19-7.22 (3H, m, Ar-**H** (Phe)), 7.04 (2H, dd J=1.3Hz, J=6.7Hz, Ar-**H** (Phe)), 6.86-6.92 (2H, m, Ar-**H** (4-F-Ph)), 5.73-5.85 (2H, m, NCH$_2$C**H**CHCH$_2$ and NCH$_2$CHC**H**CH$_2$), 4.93-4.97 (1H, m, C**H**CH$_2$Ph), 4.14 (1H, ddd J=2.1Hz, J=4.6Hz, J=6.1Hz, NCH$_2$CHCHC**H**$_2$), 3.83-3.89 (1H, m, NCH$_2$CHCHC**H**$_2$), 3.69 (3H, s, CO$_2$C**H**$_3$), 3.08-3.12 (1H, m, C**H**CHCO$_2$CH$_3$), 2.88-2.92 (2H, m, CHC**H**$_2$Ph), 2.56-2.60 (2H, m, NC**H**$_2$CHCHCH$_2$)

^{13}C NMR ppm (75 MHz in CDCl$_3$). 173.2, 138.0, 134.9, 134.2, 130.5, 130.4, 129.4, 128.6, 126.7, 118.0, 117.5, 115.8, 115.5, 62.1, 51.7, 49.9, 48.5, 37.5, 34.5

HRMS (ES) 404.1320 (MH$^+$). $C_{21}H_{22}FNO_4S$ requires 404.1332

(S)-2-[(S)-1-(4-Fluorobenzenesulfonylamino)-2-phenylethyl]pent-4-enoic acid (3.64)



Methyl ester **3.60** (0.200g, 0.511 mmol) was hydrolysed using General Procedure C2 to afford a yellow solid, 0.0887g, 46%.

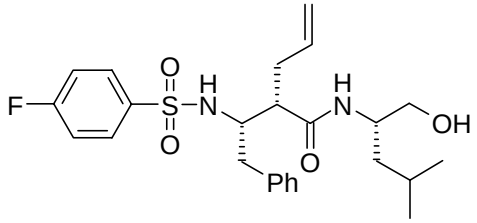
^{1}H NMR ppm (300 MHz in CDCl$_3$) 7.74-7.82 (2H, m, Ar-**H** (4-F-Ph)), 7.03-7.25 (7H, m, Ar-**H** (4-F-Ph) and Ar-**H** (Phe)), 5.81 (1H, d J=9.4Hz, N**H**), 5.52-5.67 (1H, m, CH$_2$C**H**CH$_2$), 4.92-5.03 (2H, m, CH$_2$CHC**H**$_2$), 3.68-3.78 (1H, m, C**H**CH$_2$Ph), 2.75-2.81 (2H, m, CHC**H**$_2$Ph), 2.65 (1H, ddd J=3.1Hz, J=7.1Hz, J=7.1Hz, C**H**CO$_2$CH$_3$),

2.45 (1H, ddd J=7.0Hz, J=7.1Hz, J=14.2Hz, C**H**$_2$CHCH$_2$), 2.26 (1H, ddd J=7.1Hz, J=7.2Hz, J=14.2Hz, C**H**$_2$CHCH$_2$)

^{13}C NMR ppm (75 MHz in CDCl$_3$). 178.7, 166.5, 163.2, 137.1, 136.7, 134.1, 129.6, 129.4, 129.1, 128.7, 128.4, 126.9, 117.8, 116.4, 116.1, 55.9, 46.2, 40.5, 33.2

LRMS (ES) 378.1 (MH$^+$). $C_{19}H_{20}FNO_4S$ requires 378.1

(S)-2-[(S)-1-(4-Fluorobenzenesulfonylamino)-2-phenylethyl]pent-4-enoic acid ((S)-1-hydroxymethyl-3-methylbutyl)amide (3.65)

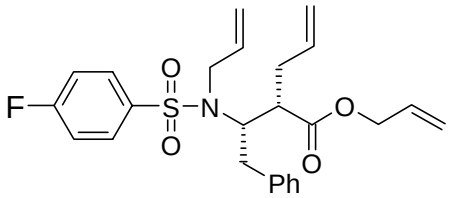


Carboxylic acid **3.64** (0.180g, 0.477 mmol) was reacted with (L)-leucinol using General Procedure B3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.125g, 61%. R_f = 0.20 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD$_3$OD) 7.62-7.67 (2H, m, Ar-**H** (4-F-Ph), 7.06-7.10 (3H, m, Ar-**H** (4-F-Ph) and Ar-**H** (Phe)), 6.96-7.03 (4H, m, Ar-**H** (Phe)), 5.59-5.70 (1H, m, CH$_2$C**H**CH$_2$), 4.89-4.98 (2H, m, CH$_2$CHC**H**$_2$), 3.94-4.02 (1H, m, C**H**CHCH$_2$(CH$_3$)$_2$), 3.68 (1H, ddd J=6.3Hz, J=6.6Hz, J=9.8Hz, C**H**CH$_2$Ph), 3.45-3.49 (2H, m, CHC**H**$_2$OH), 2.82 (1H, dd J=6.6Hz, J=14.0Hz, CHC**H**$_2$Ph), 2.75 (1H, dd J=6.3Hz, J=14.0Hz, CHC**H**$_2$Ph), 2.51-2.62 (1H, m, C**H**CH$_2$CHCH$_2$), 2.17-2.39 (2H, m, CHC**H**$_2$CHCH$_2$), 1.58-1.65 (1H, m, CHC**H**CH$_2$(CH$_3$)$_2$), 1.24-1.38 (2H,m, CHCHC**H**$_2$(CH$_3$)$_2$), 0.89 (3H, d J=6.6Hz, CHCHCH$_2$(C**H**$_3$)$_2$), 0.86 (3H, d J=6.6Hz, CHCHCH$_2$(C**H**$_3$)$_2$

HRMS (ES) 477.2221 (MH$^+$). $C_{25}H_{33}FN_2O_4S$ requires 477.2223

(S)-2-{(S)-1-[Allyl-(4-fluorobenzenesulfonyl)amino]-2-phenylethyl}pent-4-enoic acid allyl ester (3.66)

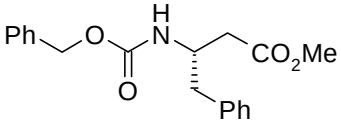


Sulfonamide **3.65** (0.120g, 0.280 mmol) was allylated using General Procedure O2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.110g, 84%. R_f = 0.51 (1/9 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.40-7.46 (2H, m, Ar-**H** (4-F-Ph)), 7.26-7.30 (3H, m, Ar-**H** (Phe)), 7.20-7.22 (2H, m, Ar-**H** (4-F-Ph)), 6.93-6.98 (2H, m, Ar-**H** (Phe)), 5.87-5.96 (1H, m, $OCH_2C\mathbf{H}CH_2$), 5.73 (1H, dddd J=6.9Hz, J=6.9Hz, J=10.0Hz, J=13.7Hz, $NCH_2C\mathbf{H}CH_2$), 5.51 (1H, tdd, J=6.4Hz, J=6.4Hz, J=9.7Hz, J=13.1Hz, $CHCH_2C\mathbf{H}CH_2$), 5.02-5.35 (6H, m, $OCH_2CHC\mathbf{H_2}$ and $NCH_2CHC\mathbf{H_2}$ and $CHCH_2CHC\mathbf{H_2}$), 4.49-4.60 (3H, m, $OC\mathbf{H_2}CHCH_2$ and $C\mathbf{H}CH_2Ph$), 3.81 (1H, dd, J=6.9Hz, J=16.1Hz, $NC\mathbf{H_2}CHCH_2$), 3.73 (1H, dd J=6.9Hz, J=16.1Hz, $NC\mathbf{H_2}CHCH_2$), 3.07 (1H, dd J=5.6Hz, J=14.4Hz, $C\mathbf{H}CH_2Ph$), 3.01 (1H, dd J=8.5Hz, J=14.4Hz, $C\mathbf{H}CH_2Ph$), 2.84-2.90 (1H, m, $C\mathbf{H}CH_2CHCH_2$), 2.18-2.29 (2H, m, $CHC\mathbf{H_2}CHCH_2$)

HRMS (ES) 458.1792 (MH^+). $C_{25}H_{28}FNO_4S$ requires 458.1801

(S)-3-Benzyloxycarbonylamino-4-phenylbutyric acid methyl ester (3.67) Lit[6]



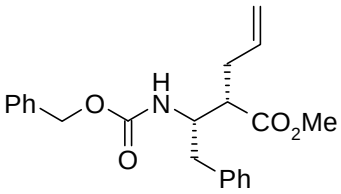
N-CBZ-Phe-H (5.00g, 16.7 mmol) was homologated using General Procedure P. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 2.62g, 48%. R_f = 0.48 (1/3 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.08-7.34 (10H, m, Ar-**H**), 5.41 (1H, d J=8.6Hz, N**H**), 5.05 (2H, s, $OC\mathbf{H_2}Ph$), 4.15-4.28 (1H, m, $C\mathbf{H}CH_2Ph$), 3.63 (3H, s, $CO_2C\mathbf{H_3}$), 2.94 (1H, dd J=6.5Hz, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.81 (1H, dd J=7.6Hz, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.49 (2H, dd J=5.3Hz, J=5.3Hz, $C\mathbf{H_2}CO_2CH_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.0, 155.7, 137.5, 136.5, 135.7, 133.2, 129.3, 128.5, 128.5, 128.1, 128.0, 126.7, 66.6, 51.7, 49.4, 40.2, 37.4

LRMS (ES) 328.1 (MH^+). $C_{19}H_{21}NO_4$ requires 328.2

(S)-2-((S)-1-tert-Butoxycarbonylamino-2-phenylethyl)pent-4-enoic acid methyl ester (3.68)



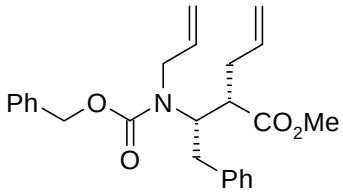
β-amino acid **3.67** (1.00g, 3.05 mmol) was allylated using General Procedure Q. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield an orange solid, 0.730g, 65%. R_f = 0.42 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.18-7.40 (10H, m, Ar-**H**), 5.84 (1H, d J=9.6Hz, N**H**), 5.62-5.72 (1H, m, $CHCH_2C\mathbf{H}CH_2$), 4.94-5.17 (2H, m, $CHCH_2CHC\mathbf{H_2}$), 4.06-4.17 (1H, m, $C\mathbf{H}CH_2Ph$), 3.71 (3H, s, $CO_2C\mathbf{H_3}$), 2.92 (1H, dd J=6.3Hz, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.70 (1H, dd J=8.5Hz, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.60-2.66 (1H, m, $CHC\mathbf{H_2}CHCH_2$), 2.38-2.47 (1H, m, $CHC\mathbf{H_2}CHCH_2$), 2.30 (1H, ddd J=6.9Hz, J=6.9Hz, J=13.8Hz, $C\mathbf{H}CO_2CH_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 174.7, 155.9, 137.4, 136.6, 134.3, 129.2, 128.4, 127.9, 127.9, 126.5, 117.4, 66.5, 53.1, 51.6, 46.4, 40.5, 34.3

LRMS (ES) 368.2 (MH^+). $C_{22}H_{25}NO_4$ requires 368.2

(S)-2-((S)-1-Benzyloxycarbonylamino-2-phenylethyl)pent-4-enoic acid methyl ester (3.69)



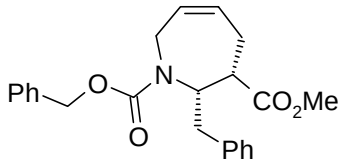
β-amino acid **3.68** (0.960g, 2.61 mmol) was allylated using General Procedure O3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.926g, 87%. R_f = 0.22 (1/7 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.02-7.37 (10H, m, Ar-**H**), 5.68-5.82 (2H, m $OCH_2C\mathbf{H}CH_2$ and $CHOCH_2C\mathbf{H}CH_2$, 5.01-5.16 (6H, m, $OCH_2CHC\mathbf{H_2}$ and $CHCH_2CHC\mathbf{H_2}$ and $OC\mathbf{H_2}Ph$), 3.84-3.90 (1H, m, $C\mathbf{H}CH_2Ph$), 3.56 (3H, s, $CO_2C\mathbf{H_3}$), 3.35-3.46 (2H, m, $NC\mathbf{H_2}CHCH_2$), 2.87-2.95 (2H, m, $CHC\mathbf{H_2}Ph$), 2.38-2.45 (3H, m, $C\mathbf{H}CH_2CHCH_2$ and $CHC\mathbf{H_2}CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 174.7, 155.9, 137.4, 136.6, 134.3, 129.2, 128.4, 127.9, 126.5, 117.4, 66.5, 53.1, 51.6, 46.4, 40.5, 34.3

LRMS (ES) 408.2 (MH^+). $C_{25}H_{29}NO_4$ requires 408.2

(2S,3S)-2-Benzyl-2,3,4,7-tetrahydro-azepine-1,3-dicarboxylic acid 1-benzyl ester-3-methyl ester (3.70)

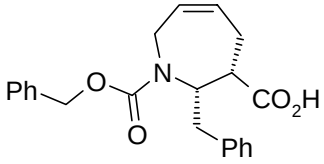


Diene **3.69** (0.930g, 2.28 mmol) was reacted with Grubbs second generation catalyst using General Procedure D1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.710g, 82%. R_f = 0.29 (1/5 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.13-7.33 (10H, m, Ar-**H**), 5.71-5.80 (2H, m, $NCH_2C\mathbf{H}CHCH_2$ and $NCH_2CHC\mathbf{H}CH_2$), 5.26-5.33 (1H, m, $C\mathbf{H}CH_2Ph$), 5.10 (2H, s, $OC\mathbf{H_2}Ph$), 4.45-4.51 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 4.24-4.31 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 3.71-3.77 (1H, m, $C\mathbf{H}CO_2CH_3$), 3.64 (3H, s, $CO_2C\mathbf{H_3}$), 3.01-3.04 (1H, m, $CHC\mathbf{H_2}Ph$), 2.89-2.95 (1H, m, $CHC\mathbf{H_2}Ph$), 2.53-2.67 (2H, m, $NC\mathbf{H_2}CHCHCH_2$)

HRMS (ES) 380.1871 (MH^+). $C_{23}H_{25}NO_4$ requires 380.1862

(2S,3S)-2-Benzyl-2,3,4,7-tetrahydro-azepine-1,3-dicarboxylic acid-1-benzyl ester (3.71)



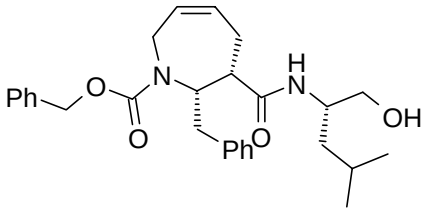
Methyl ester **3.70** (0.710g, 1.87 mmol) was hydrolysed using General Procedure C1 to afford an orange solid, 0.683g, 100%.

^{1}H NMR ppm (300 MHz in $CDCl_3$) 7.02-7.46 (10H, m, Ar-**H**), 5.63-5.88 (2H, m, $NCH_2C\mathbf{H}CHCH_2$ and $NCH_2CHC\mathbf{H}CH_2$) 5.28-5.33 (1H, m, $C\mathbf{H}CH_2Ph$), 5.10 (2H, s, $OC\mathbf{H_2}Ph$), 4.45-4.52 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 4.20-4.29 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 3.71-3.79 (1H, m, $C\mathbf{H}CO_2H$), 3.06-3.12 (1H, m, $CHC\mathbf{H_2}Ph$), 2.92-3.00 (1H, m, $CHC\mathbf{H_2}Ph$), 2.57-2.64 (2H, m, $NC\mathbf{H_2}CHCHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 180.5, 137.6, 129.4, 129.3, 128.6, 128.5, 128.4, 127.9, 126.5, 127.8, 127.5, 67.3, 56.5, 47.6, 47.1, 41.4, 32.9, 26.5

LRMS (ES) 366.1 (MH^+). $C_{22}H_{23}NO_4$ requires 366.2

(2S,3S)-2-Benzyl-3-((S)-1-hydroxymethyl-3-methylbutylcarbamoyl)-2,3,4,7-tetra-hydroazepine-1-carboxylic acid benzyl ester (3.72)

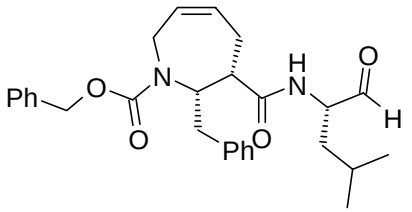


Carboxylic acid **3.71** (0.650g, 1.78 mmol) was reacted with (L)-leucinol using General Procedure B3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.481g, 58%. R_f = 0.43 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.18-7.37 (10H, m, Ar-**H**), 6.30 (1H, bs, N**H**), 5.63-5.78 (2H, m, $NCH_2C\mathbf{H}CHCH_2$ and $NCH_2CHC\mathbf{H}CH_2$), 5.02 (2H, s, $OC\mathbf{H_2}Ph$), 4.93-4.97 (1H, m, $C\mathbf{H}CH_2Ph$), 4.57-4.61 (1H, m, $CHC\mathbf{H}C(O)$, 4.23-4.29 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 4.01-4.07 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 3.64-3.72 (2H, m, $NCH_2CHCHC\mathbf{H_2}$ and $C\mathbf{H_2}OH$), 3.48-3.52 (1H,m, $C\mathbf{H_2}OH$), 2.88-3.05 (2H, m $CHC\mathbf{H_2}Ph$), 2.68-2.74 (1H, m, $NC\mathbf{H_2}CHCHCH_2$), 2.36-2.43 (1H, m, $NC\mathbf{H_2}CHCHCH_2$), 1.62-1.69 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 1.36-1.52 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 0.88-0.92 (6H, m, $CHCH_2CH(C\mathbf{H_3})_2$)

HRMS (ES) 465.2756 (MH^+). $C_{28}H_{36}N_2O_4$ requires 465.2753

(2S,3S)-2-Benzyl-3-((S)-1-formyl-3-methylbutylcarbamoyl)-2,3,4,7-tetrahydroazepine-1-carboxylic acid benzyl ester (3.73)

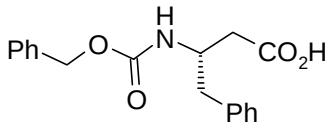


Alcohol **3.72** (0.520g, 1.11 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 0.314g, 61%. R_f = 0.33 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$). Compound **3.73** exists as a 1:1 mixture of rotamers. 9.44 and 9.40 (1H, s, C**H**O), 8.38 and 8.28 (1H, d, J=7.0Hz, N**H**), 7.09-7.38 (10H, m, Ar-**H**), 5.70-5.85 (2H, m, $NCH_2C\mathbf{H}CHCH_2$ and $NCH_2CHC\mathbf{H}CH_2$), 5.03 (1H, d J=13.1Hz, $OC\mathbf{H_2}Ph$), 5.02 (1H, d J=13.1Hz, $OC\mathbf{H_2}Ph$), 4.91-4.98 (1H, m, $C\mathbf{H}CH_2Ph$), 4.28-4.36 and 4.20-4.26 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 4.06-4.19 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 3.72-3.94 (1H, m, $NCH_2CHCHC\mathbf{H_2}$), 2.93-3.17 (2H, m C**H**CHO and $CHC\mathbf{H_2}Ph$), 2.55-2.83 (2H, m, $CHC\mathbf{H_2}Ph$ and $NC\mathbf{H_2}CHCHCH_2$), 2.24-2.39 (1H, m, $NC\mathbf{H_2}CHCHCH_2$), 1.59-1.73 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 1.40-1.59 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 0.84-0.91 (6H, m, $CHCH_2CH(C\mathbf{H_3})_2$)

HRMS (ES) 463.2602 (MH^+). $C_{28}H_{34}N_2O_4$ requires 463.2597

(S)-3-Benzyloxycarbonylamino-4-phenylbutyric acid (3.74) Lit[6]

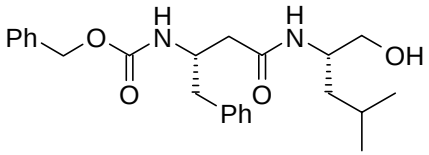


Methyl ester **3.67** (2.00g, 6.11 mmol) was hydrolysed using general procedure C1 to afford a white solid, 1.80g, 94%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.29 (1H, bs, $CO_2\mathbf{H}$), 7.07-7.39 (10H, m, Ar-**H** (CBZ) and Ar-**H** (Phe)), 5.32 (1H, d J=8.4Hz, N**H**), 5.07 (2H, s, $OC\mathbf{H_2}Ph$), 4.16-4.31 (1H, m, $C\mathbf{H}CH_2Ph$), 2.96 (1H, dd J=6.4Hz, J=13.5Hz, $C\mathbf{H}CH_2Ph$), 2.87 (1H, dd J=7.9Hz, J=13.5Hz, $C\mathbf{H}CH_2Ph$), 2.59 (1H, dd J=5.0Hz, J=16.3Hz $C\mathbf{H_2}CO_2H$), 2.53 (1H, dd J=5.1Hz, J=16.3Hz, $C\mathbf{H_2}CO_2H$)

LRMS (ES) 314.1 (MH^+). $C_{18}H_{19}NO_4$ requires 314.1

(S)-1-Benzyl-2-((S)-1-hydroxymethyl-3-methylbutylcarbamoyl)ethyl]carbamic acid benzyl ester (3.75)



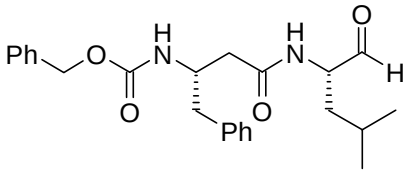
Carboxylic acid **3.74** (1.80g, 5.74 mmol) was reacted with (L)-leucinol using General Procedure B3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.711g, 30%. R_f = 0.29 (3/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 7.17-7.32 (10H, m, Ar-**H** (CBZ) and Ar-**H** (Phe)), 4.99 (1H, d J=12.7Hz OC**H$_2$**Ph), 4.93 (1H, d J=12.7Hz OC**H$_2$**Ph), 4.12-4.19 (1H, m, C**H**CH_2Ph), 3.90-3.98 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.42 (1H, dd J=5.5Hz, J=11.0Hz, C**H$_2$**OH), 3.39 (1H, dd J=5.5Hz, J=11.0Hz, C**H$_2$**OH), 2.84 (1H, dd J=5.9Hz, J=13.5Hz, CHC**H$_2$**Ph), 2.77 (1H, dd J=8.2Hz, J=13.5Hz, CHC**H$_2$**Ph), 2.34-2.44 (2H, m, CHC**H$_2$**C(O)), 1.58-1.66 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.33-1.36 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 0.88 (3H, d J=6.7Hz, $CHCH_2CH(CH_3)_2$), 0.86 (3H, d J=6.7Hz, $CHCH_2CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 171.8, 156.7, 138.2, 137.0, 129.2, 128.6, 128.1, 128.0, 127.5, 127.3, 126.1, 65.9, 64.3, 50.4, 49.4, 49.3, 40.8, 40.4, 39.7, 24.6, 22.5, 20.9

HRMS (ES) 413.2439 (MH^+). $C_{17}H_{27}N_2O_2$ requires 413.2440

[(S)-1-Benzyl-2-((S)-1-formyl-3-methylbutylcarbamoyl)ethyl]carbamic acid benzyl ester (3.76)



Alcohol **3.75** (0.150g, 0.364 mmol) was oxidised using General Procedure L to afford a white solid, 0.0776g, 52%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.55 (1H, s, C**H**O), 7.13-7.29 (10H, m, Ar-**H** (CBZ) and Ar-**H** (Phe)), 6.05 (1H, d J=4.9Hz, N**H** Leu), 5.68 (1H, d J=6.7Hz, N**H** Phe), 5.07 (2H, s, OC**H$_2$**Ph), 4.47-4.51 (1H, m, C**H**$CH_2CH(CH_3)_2$), 4.13-4.20 (1H, m, C**H**CH_2Ph), 3.01 (1H, dd J=6.4Hz, J=12.8, CHC**H$_2$**Ph), 2.85 (1H, dd J=8.0Hz, J=12.8Hz, CHC**H$_2$**Ph), 2.33-2.45 (2H, m, CHC**H$_2$**C(O)), 1.57-1.64 (2H, m, CHC**H$_2$**$CH(CH_3)_2$), 1.30-1.35 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 0.93-0.96 (6H, m, $CHCH_2CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 199.1, 171.1, 155.9, 137.7, 136.4, 129.3, 128.6, 128.5, 128.0, 127.9, 126.7, 66.6, 57.2, 50.2, 40.2, 38.8, 37.7, 24.8, 23.0, 21.8

HRMS (ES) 411.2289 (MH^+). $C_{24}H_{30}N_2O_4$ requires 411.2284

References for Chapter 3 Experimental

1. Reichwein, J. F.; Liskamp, R. M. J., Synthesis of cyclic dipeptides by ring-closing metathesis. *European Journal of Organic Chemistry* **2000**, (12), 2335-2344.

2. Payne, R. J.; Brown, K. M.; Coxon, J. M.; Morton, J. D.; Lee, H. Y.-Y.; Abell, A. D., Peptidic Aldehydes Based on alpha and beta-Amino Acids: Synthesis, Inhibition of m-Calpain, and Anti-Cataract Properties. *Australian Journal of Chemistry* **2004,** 57, (9), 877-884.

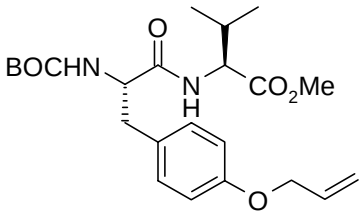
3. Spezzacatena, C.; Perri, T.; Guantieri, V.; Sandberg, L. B.; Mitts, T. F.; Tamburro, A. M., Classical synthesis of and structural studies on a biologically active heptapeptide and a nonapeptide of bovine elastin. *European Journal of Organic Chemistry* **2002**, (1), 95-103.

4. Chakraborty, T. K.; Ghosh, A., A convenient synthesis of chiral beta-3-amino acids. *Synlett* **2002**, (12), 2039-2040.

5. Gopi, H. N.; Roy, R. S.; Raghothama, S. R.; Karle, I. L.; Balaram, P., b-hairpins generated from hybrid peptide sequences containing both alpha and beta-amino acids. *Helvetica Chimica Acta* **2002,** 85, (10), 3313-3330.

6. Caputo, R.; Cassano, E.; Longobardo, L.; Palumbo, G., Amino acid and peptide chemistry. 3. Synthesis of enantiopure N- and C-protected homo-beta-amino acids by direct homologation of alpha-amino acids. *Tetrahedron* **1995,** 51, (45), 12337-50.

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tert-butoxycarbonylaminopropionylamino]-3-methylbutyric acid methyl ester (4.12)



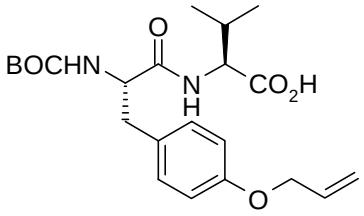
N-BOC-O-allyl-Tyr-H (5.00g, 16.3 mmol) was reacted with Val-OMe using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 6.08g, 69%. R_f = 0.29 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 in $CDCl_3$) 7.09 (2H, d J=6.5Hz, Ar-**H**), 6.80 (2H, d J=6.5Hz, Ar-**H**), 6.47 (1H, bs, N**H** Val), 5.97-6.05 (1H, m, $OCH_2C\mathbf{H}CH_2$), 5.01-5.40 (2H, m, $OCH_2CHC\mathbf{H_2}$), 5.12 (1H, bs, N**H** Tyr), 4.47-4.49 (2H, m, $OC\mathbf{H_2}CHCH_2$), 4.37-4.46 (1H, m, $C\mathbf{H}CH_2Ph$), 4.30-4.34 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.66 (3H, s, $CO_2C\mathbf{H_3}$), 3.01-3.04 (2H, m, $CHC\mathbf{H_2}Ph$), 2.04-2.11 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.39 (9H, s, $C(C\mathbf{H_3})_3$), 0.85 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$), 0.82 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 174.7, 171.9, 157.5, 133.2, 130.3, 128.6, 117.6, 114.8, 68.7, 57.2, 37.0, 31.0, 28.2, 18.8, 17.6

HRMS (ES) 435.2501 (MH^+). $C_{23}H_{34}N_2O_6$ requires 435.2495

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-methylbutyric acid (4.13)

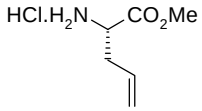


Dipeptide **4.12** (4.90g, 11.3 mmol) was dissolved in THF (70 mL) and hydrolysed using General procedure C1. This afforded a white solid, 4.60g, 97%.

^{1}H NMR ppm (500 in $CDCl_3$) 7.11 (2H, d J=8.3Hz, Ar-**H**), 6.83 (2H, d J=8.3Hz, Ar-**H**), 6.64 (1H, d J=8.2Hz, N**H** Val), 5.98-6.07 (1H, m, $OCH_2C\mathbf{H}CH_2$), 5.00-5.39 (2H, m, $OCH_2CHC\mathbf{H_2}$), 5.19 (1H, bs, N**H** Tyr), 4.51 (3H, m, $C\mathbf{H}CH_2Ph$ and $OC\mathbf{H_2}CHCH_2$), 4.36-4.40 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 2.97-3.02 (2H, m, $CHC\mathbf{H_2}Ph$), 2.16-2.23 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.40 (9H, s, $C(C\mathbf{H_3})_3$), 0.88-0.93 (6H, m, $CHCH(C\mathbf{H_3})_2$)

LRMS (ES) 421.3 (MH^+). $C_{22}H_{32}N_2O_6$ requires 421.2

(S)-2-Amino-pent-4-enoic acid methyl ester (4.14) Lit[1]



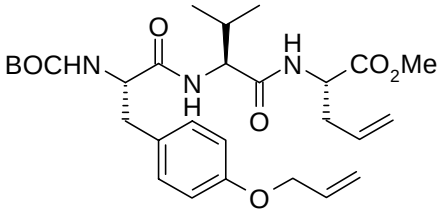
(s)-allyl-Gly-H (5.00g, 43.4 mmol) was suspended in Methanol (100 mL). This was esterified using General procedure F1 to afford a white solid. 7.19g, 100%.

^{1}H NMR ppm (500 MHz in CD_3OD) 5.78 (1H, dddd J=7.1Hz, J=7.2Hz, J=7.2Hz, J=10.0Hz, $CH_2C\mathbf{H}CH_2$), 5.25-5.31 (2H, m, $CH_2CHC\mathbf{H_2}$), 4.16 (1H, dd J=6.2Hz, J=6.2Hz, $C\mathbf{H}CO_2CH_3$), 3.84 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.69 (2H, m, $C\mathbf{H_2}CHCH_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 169.1, 130.3, 120.3, 52.3, 52.1, 34.4

LRMS (ES) (MH^+). $C_6H_{11}NO_2$ requires 130.1

(S)-2-{(S)-2-[(S)-3-(4-Allyloxy-phenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-methylbutyryl-amino}-pent-4-enoic acid methyl ester (4.15)



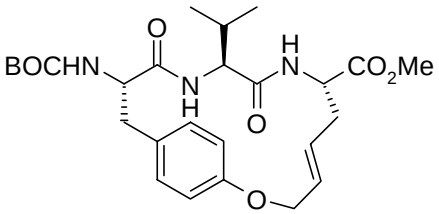
Dipeptide **4.13** (1.00g, 2.38 mmol) was reacted with amine **4.14** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 1.05g, 83%. R_f = 0.48 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 in $CDCl_3$) 7.09 (2H, d J=8.6Hz, Ar-**H**), 6.82 (2H, d J=8.6Hz, Ar-**H**), 6.61 (1H, d J=8.4Hz, N**H** Val), 6.56 (1H, d J=6.8Hz, N**H** Gly), 6.03 (1H, tdd J=5.3Hz, J=10.6Hz, J=17.2Hz, $OCH_2C\mathbf{H}CH_2$), 5.62-5.71 (1H, m, $CHCH_2C\mathbf{H}CH_2$), 5.10-5.41 (4H, m, $OCH_2CHC\mathbf{H_2}$ and $CHCH_2CHC\mathbf{H_2}$), 5.05 (1H, d J=5.5Hz, N**H** Tyr), 4.58-4.62 (1H, m, $C\mathbf{H}CH_2CHCH_2$), 4.48-4.50 (2H, m, $OC\mathbf{H_2}CHCH_2$), 4.30-4.33 (1H, m, $C\mathbf{H}CH_2Ph$), 4.25 (1H, dd J=6.5Hz, J=8.4Hz, $C\mathbf{H}CH(CH_3)_2$), 3.73 (3H, s, $CO_2C\mathbf{H_3}$), 2.96-3.05 (2H, m, $CHC\mathbf{H_2}Ph$), 2.46-2.59 (2H, m, $CHC\mathbf{H_2}CHCH_2$), 2.07-2.14 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.39 (9H, s, $C(C\mathbf{H_3})_3$), 0.90 (3H, d, J=6.8 Hz, $CHCH(C\mathbf{H_3})_2$), 0.86 (3H, d, J=6.8 Hz, $CHCH(C\mathbf{H_3})_2$)

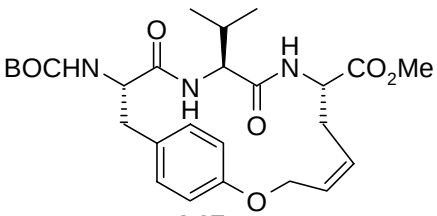
^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.7, 171.4, 170.4, 157.6, 133.2, 132.2, 130.0, 128.6, 119.3, 117.6, 114.9, 68.7, 58.4, 52.3, 51.7, 36.2, 30.7, 28.2, 19.0

HRMS (ES) 532.3010 (MH^+). $C_{28}H_{42}N_3O_7$ requires 532.3023

(*E*)-(7S,10S,13S)-13-tertButoxycarbonylamino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (4.16)



4.16



4.17

Diene **4.15** (1.80g, 3.39 mmol) was reacted with Grubbs second generation catalyst using General procedure D2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield the *E*-isomer as a brown solid, 0.205g, 12%. R_f = 0.24 (1/1 (EtOAc / (50/70) Pet ether)) and the *Z*-isomer as a brown solid, 0.528g, 31%. R_f = 0.22 (1/1 (EtOAc / (50/70) Pet ether)).

***E*-Isomer**

^{1}H NMR ppm (500 in $CDCl_3$) 7.05 (2H, d J=8.6Hz, Ar-**H**), 6.75 (2H, d J=8.6Hz, Ar-**H**), 5.74-5.75 (2H, m, N**H** Val and N**H** Gly), 5.43-5.56 (2H, m, $OCH_2C\mathbf{H}CHCH_2$ and $OCH_2CHC\mathbf{H}CH_2$), 5.32 (1H, d J=8.7Hz, N**H** Tyr), 4.76 (1H, ddd J=3.4Hz, J=9.2Hz, J=10.1Hz, $C\mathbf{H}CO_2CH_3$), 4.58-4.64 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.21 (1H, ddd J=5.2Hz, J=8.7Hz, J=11.6Hz, $C\mathbf{H}CH_2Ph$), 3.99 (1H, dd J=4.8Hz, J=7.5Hz, $C\mathbf{H}CH(CH_3)_2$), 3.75 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.13 (1H, dd J=5.2Hz, J=12.5Hz, $CHC\mathbf{H_2}Ph$), 2.66-2.75 (2H, m, $CHC\mathbf{H_2}Ph$ and $OCH_2CHCHC\mathbf{H_2}$), 2.26-2.32 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 2.07-2.14 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.45 (9H, s, $C(C\mathbf{H_3})_3$), 0.81-0.83 (6H, m, $CHCH(C\mathbf{H_3})_2$)

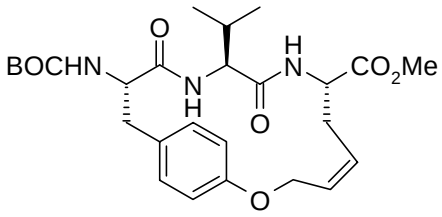
HRMS (ES) 504.2718 (MH^+). $C_{26}H_{37}N_3O_7$ requires 504.2710

***Z*-Isomer**

^{1}H NMR ppm (500 in $CDCl_3$) 7.06 (2H, d J=7.9Hz, Ar-**H**), 6.74 (2H, d J=7.9Hz, Ar-**H**), 6.14 (1H, d J=8.7Hz, N**H** Gly), 5.94 (1H, d J=7.7Hz, N**H** Val), 5.44-5.55 (2H, m, $OCH_2C\mathbf{H}CHCH_2$ and $OCH_2CHC\mathbf{H}CH_2$), 4.75 (1H, dt J=3.3Hz, J=10.6Hz, $C\mathbf{H}CO_2CH_3$), 4.55-4.65 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.24 (1H, ddd J=5.0Hz, J=8.3Hz, J=12.6Hz, $C\mathbf{H}CH_2Ph$), 4.07 (1H, dd J=5.2Hz, J=7.7Hz, $C\mathbf{H}CH(CH_3)_2$), 3.73 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.10 (1H, dd J=5.0Hz, J=12.8Hz, $CHC\mathbf{H_2}Ph$), 2.66-2.73 (2H, m, $CHC\mathbf{H_2}Ph$ and $OCH_2CHCHC\mathbf{H_2}$), 2.25-2.32 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 2.04-2.09 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.44 (9H, s, $C(C\mathbf{H_3})_3$), 0.81 (6H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 504.2718 (MH^+). $C_{26}H_{37}N_3O_7$ requires 504.2710

(*Z*)-(7S,10S,13S)-13-tertButoxycarbonylamino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (4.17)

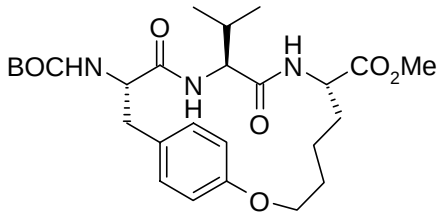


Diene **4.15** (3.50g, 6.58 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 2.25g, 31%. R_f = 0.22 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 in $CDCl_3$) 7.06 (2H, d J=7.9Hz, Ar-**H**), 6.74 (2H, d J=7.9Hz, Ar-**H**), 6.14 (1H, d J=8.7Hz, N**H** Gly), 5.94 (1H, d J=7.7Hz, N**H** Val), 5.44-5.55 (2H, m, $OCH_2C\mathbf{H}CHCH_2$ and $OCH_2CHC\mathbf{H}CH_2$), 4.75 (1H, dt J=3.3Hz, J=10.6Hz, $C\mathbf{H}CO_2CH_3$), 4.55-4.65 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.24 (1H, ddd J=5.0Hz, J=8.3Hz, J=12.6Hz, $C\mathbf{H}CH_2Ph$), 4.07 (1H, dd J=5.2Hz, J=7.7Hz, $C\mathbf{H}CH(CH_3)_2$), 3.73 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.10 (1H, dd J=5.0Hz, J=12.8Hz, $CHC\mathbf{H_2}Ph$), 2.66-2.73 (2H, m, $CHC\mathbf{H_2}Ph$ and $OCH_2CHCHC\mathbf{H_2}$), 2.25-2.32 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 2.04-2.09 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.44 (9H, s, $C(C\mathbf{H_3})_3$), 0.81 (6H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 504.2711 (MH^+). $C_{26}H_{37}N_3O_7$ requires 504.2710

(7S,10S,13S)-13-tertButoxycarbonylamino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (4.18)



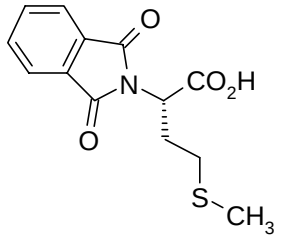
A mixture of the unsaturated macrocycles **4.16 and 4.17** (1.36g, 2.70 mmol) was hydrogenated in 50 mL of methanol using General Procedure G1 to afford a brown solid, 1.36g, 100%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.05 (2H, d J=8.0Hz, Ar-**H**), 6.78 (2H, d J=8.0Hz, Ar-**H**), 6.23 (1H, d J=7.1Hz, N**H** Val), 5.90 (1H, d J=8.2Hz, N**H** Gly), 5.29 (1H, d J=8.6Hz, N**H** Tyr), 4.56 (1H, dt J=3.9Hz, J=8.2Hz, $C\mathbf{H}CO_2CH_3$), 4.21-4.30 (2H, m, $C\mathbf{H}CH_2Ph$, $OC\mathbf{H_2}CH_2CH_2CH_2$) 4.09-4.13 (1H, m, $OC\mathbf{H_2}CH_2CH_2CH_2$), 3.84-3.86 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.72 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.10 (1H, dd J=5.4Hz, J=12.2Hz, $CHC\mathbf{H_2}Ph$), 2.67 (1H, dd J=12.2Hz, J=12.2Hz, $CHC\mathbf{H_2}Ph$), 1.95-2.02 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.86-1.92 (1H, m, $OCH_2CH_2CH_2C\mathbf{H_2}$), 1.80 (1H, m, $OCH_2C\mathbf{H_2}CH_2CH_2$), 1.49-1.57 (2H, m, $OCH_2CH_2CH_2C\mathbf{H_2}$ and $OCH_2C\mathbf{H_2}CH_2CH_2$), 1.44 (9H, s, $C(C\mathbf{H_3})_3$), 1.25-1.35 (2H, m, $OCH_2CH_2C\mathbf{H_2}CH_2$), 0.87 (3H, d J=6.6 Hz, $CHCH(C\mathbf{H_3})_2$), 0.81 (3H, d J=6.6Hz, $CHCH(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 172.7, 170.3, 169.8, 156.9, 155.1, 130.0, 128.4, 115.9, 79.6, 66.8, 57.6, 56.8, 52.3, 51.0, 38.4, 32.3, 31.7, 28.2, 21.7, 18.2, 18.1

HRMS (ES) 506.2871 (MH^+). $C_{26}H_{40}N_3O_7$ requires 506.2866

(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-methylsulfanyl-butyric acid (4.19) Lit[2]



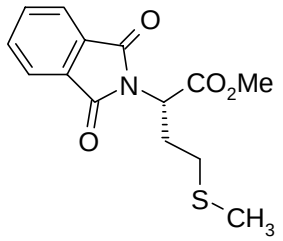
(*L*)-Methionine (5.00g, 3.35 mmol) and phthalic anhydride (4.96g, 1 equiv) were suspended in water (7 mL). This was heated in the microwave (800W) for six min in an open round bottomed flask to allow evaporation of the water. A yellow gum was obtained, 9.36g, 100%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.81 (1H, bs, CO_2**H**), 7.85 (2H, d J=5.6Hz, Ar-**H**), 7.73 (2H, d J=5.6Hz, Ar-**H**), 5.16 (1H, dd J=4.5Hz, J=8.9Hz, C**H**CO_2H), 2.44-2.60 (4H, m, CHC**H$_2$**CH_2S and $CHCH_2$C**H$_2$**S), 2.07 (3H, s, SC**H$_3$**)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 174.6, 167.6, 134.3, 131.6, 123.6, 50.6, 30.7, 27.7, 15.2

LRMS (ES) 280.1 (MH^+). $C_{13}H_{13}NO_4S$ requires 280.1

(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-methylsulfanyl-butyric acid methyl ester (4.20) Lit[2]



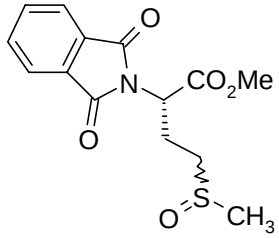
Carboxylic acid **4.19** (9.36g, 33.5 mmol) was suspended in methanol (150 mL). This was cooled in ice and saturated with hydrogen chloride gas. This was warmed to rt and stirred for eighteen h. The reaction mixture was concentrated *in vacuo* and the crude material purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to afford a colourless oil, 9.83g, 100%. R_f = 0.31 (1/3 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.88 (2H, d J=5.5Hz, Ar-**H**), 7.77 (2H, d J=5.5Hz, Ar-**H**), 5.09-5.12 (1H, m, C**H**CO_2CH_3), 3.75 (3H, s, $CHCO_2$C**H$_3$**), 2.47-2.61 (4H, m, CHC**H$_2$**CH_2S and $CHCH_2$C**H$_2$**S), 2.09 (3H, s, SC**H$_3$**)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 169.5, 167.4, 134.2, 131.7, 123.5, 52.8, 50.7, 30.7, 28.0, 15.2

LRMS (ES) 294.1 (MH^+). $C_{14}H_{15}NO_4S$ requires 294.1

(S)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-methanesulfinylbutyric acid methyl ester (4.21) Lit[2]

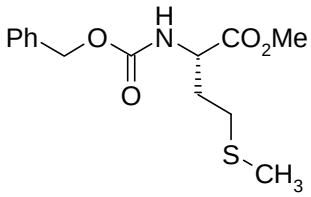


Sulphide **4.20** (1.40g, 4.78 mmol) was dissolved in methanol (30 mL). This was cooled in ice and a solution of sodium metaperiodate (1.07g, 1.05 equiv) in water (12 mL) was added dropwise over ten min. This was stirred in ice for one h and then at rt for a further two h. The resultant white precipitate was removed by filtration under vacuum, the residue washed with methanol and the filtrate concentrated *in vacuo* to yield **4.21** as a 1:1 mixture of diastereoisomers. This was obtained as a colourless oil, 1.39g, 94%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.84-7.86 (2H, m, Ar-**H**), 7.74-7.76 (2H, m, Ar-**H**), 4.93-4.97 (1H, m, C**H**CO_2CH_3), 3.76 (3H, s, $CHCO_2C$**H$_3$**), 2.60-2.88 (4H, m, CHC**H$_2$**CH_2S and $CHCH_2C$**H$_2$**S) 2.53-2.54 (3H, m, SC**H$_3$**)

LRMS (ES) 310.1 (MH^+). $C_{14}H_{15}NO_5S$ requires 310.1

(S)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyric acid methyl ester (4.23) Lit[3]

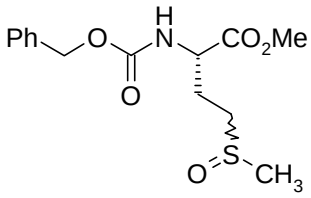


(*L*)-methionine (3.70g, 18.5 mmol) was dissolved in a biphasic mixture of EtOAc (75 mL) and water (75 mL). This was cooled in ice and sodium hydrogen bicarbonate (7.78g, 5 equiv) and benzyl chloroformate (2.98 mL, 1.1 equiv) were added. This was stirred in ice for one h and then at rt for a further eighteen h before the mixture was allowed to partition. The organic phase was washed sequentially with 1M $HCl_{(aq)}$, saturated aqueous sodium hydrogen bicarbonate and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a yellow oil, 5.40g, 98%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.29 (5H, m, Ar-**H**), 5.79 (1H, d J=7.9Hz, N**H**), 5.08 (2H, s, C**H$_2$**Ph), 4.43-4.47 (1H, m, C**H**CO_2CH_3), 3.70 (3H, s, $CHCO_2C$**H$_3$**), 2.49 (2H, dd, J=7.4Hz, J=7.4Hz, $CHCH_2C$**H$_2$**S), 2.11 (1H, ddd J=7.2Hz, J=12.8Hz, J=14.4Hz, CHC**H$_2$**CH_2S), 2.04 (3H, s, SC**H$_3$**), 1.93 (1H, ddd, J=7.1Hz, J=7.1Hz, J=14.4Hz, CHC**H$_2$**CH_2S)

LRMS (ES) 298.1 (MH^+). $C_{14}H_{19}NO_4S$ requires 298.1

(S)-2-Benzyloxycarbonylamino-4-methanesulfinylbutyric acid methyl ester (4.24) Lit[3]



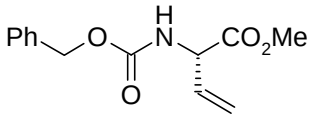
Sulphide **4.23** (5.40g, 18.2 mmol) was dissolved in methanol (60 mL). This was cooled in ice and a solution of sodium metaperiodate (4.08g, 1.05 equiv) in water (25 mL) was added dropwise over ten min. This was stirred in ice for one h and then at rt for a further two h. The resultant white precipitate was removed by filtration under vacuum, the residue washed with methanol and the filtrate concentrated *in vacuo*. The resultant colourless oil was partitioned between DCM and water. The aqueous phase was extracted twice more with DCM, methanol added to the combined organic extracts to obtain an homogenous solution and this was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a yellow oil, 5.69g, 100%. This was obtained as a 1:1 mixture of diastereoisomers.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.30-7.35 (5H, m, Ar-**H**), 6.00-6.08 (1H, m, N**H**), 5.10 (2H, s, CH_2Ph), 4.44-4.50 (1H, m, C**H**CO_2CH_3), 3.75 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.67-2.77 (2H, m, $CHCH_2C\mathbf{H_2}S$), 2.53-2.54 (3H, m, $SC\mathbf{H_3}$), 2.32-2.40 (1H, m, $CHC\mathbf{H_2}CH_2S$), 2.09-2.19 (1H, m, $CHC\mathbf{H_2}CH_2S$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 172.6, 156.0, 136.2, 128.6, 128.4, 128.3, 128.1, 128.0, 69.9, 53.5, 53.0, 52.4, 31.6, 29.8, 15.3

LRMS (ES) 314.1 (MH^+). $C_{14}H_{19}NO_5S$ requires 314.1

(S)-2-Benzyloxycarbonylamino-but-3-enoic acid methyl ester (4.25) Lit[3]



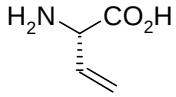
Sulfoxide **4.24** (5.69g, 18.2 mmol) was placed in a round bottomed flask in a kugelrohr. This was directly connected to a low vacuum diaphragm pump and heated/distilled at 140^{o}C. The distillate was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 0.905g, 20%. R_f = 0.24 (1/7 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.31-7.35 (5H, m, Ar-**H**), 5.86-5.93 (1H, m, C**H**CH_2), 5.61 (1H, d J=6.3Hz, N**H**), 5.01-5.39 (2H, m, $CHC\mathbf{H_2}$), 5.11 (2H, s, $C\mathbf{H_2}Ph$), 4.91-4.96 (1H, m, C**H**CO_2CH_3) , 3.74 (3H, s, $CHCO_2C\mathbf{H_3}$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 170.9, 155.5, 136.1, 132.2, 128.5, 128.2, 128.1, 127.5, 126.9, 117.7, 67.1, 56.1, 52.7

LRMS (ES) 250.1 (MH^+). $C_{13}H_{16}NO_4$ requires 250.1

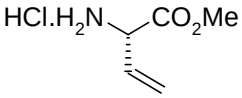
(S)-2-Benzyloxycarbonylamino-but-3-enoic acid methyl ester (4.26) Lit[4]



Olefin **4.25** (0.905g, 3.61 mmol) was suspended in 6M $HCl_{(aq)}$(20 mL) This was heated at reflux for two h, cooled and partitioned with DCM. The aqueous phase was washed again with DCM before being concentrated *in vacuo* to afford a white solid. This was recrystallised from acetone to afford a white solid, 0.350g, 71%

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 8.32 (2H, bs, N**H$_2$**), 6.02-6.11 (1H, m, C**H**CH_2), 5.50-5.75 (2H, m, CHC**H$_2$**), 4.68-4.73 (1H, m, C**H**CO_2H)

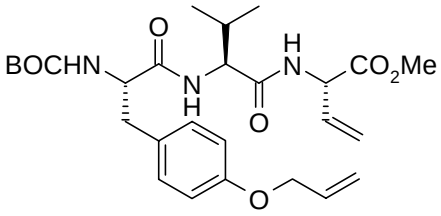
(S)-2-Amino-but-3-enoic acid methyl ester hydrochloride (4.27) Lit[4]



Amino acid **4.26** (0.350g, 2.56 mmol) was dissolved in methanol (10 mL) and esterified using General Procedure F2 to afford a white solid, 0.388g, (100%)

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.70 (2H, bs, N**H$_2$**), 6.06-6.13 (1H, m, C**H**CH_2), 5.52-5.73 (2H, m, CHC**H$_2$**), 4.79-4.82 (1H, m, C**H**CO_2CH_3), 3.83 (3H, s, $CHCO_2C$**H$_3$**)

(S)-2-((S)-2-{(S)-3-(4-Allyloxyphenyl)-2-[(tertbutoxyhydroxymethyl)amino]propionylamino}-3-methyl-butyrylamino)but-3-enoic acid methyl ester (4.28)

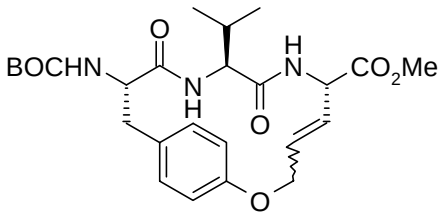


Dipeptide **4.13** (1.03g, 2.45 mmol) was reacted with amine **4.27** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.964g, 76%. R_f = 0.46 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.08 (2H, d J=8.2Hz, Ar-**H**), 6.82 (2H, d J=8.2Hz, Ar-**H**), 6.62 (1H, d J=7.5Hz, N**H**), 6.53 (1H, d J=8.5Hz, N**H**), 6.42 (1H, d J=8.3Hz, N**H**), 6.07 (1H, tdd J=5.3Hz, J=10.6Hz, J=17.2Hz, OCH_2C**H**CH_2), 5.90-5.97 (1H, m, CHCHC**H**CH_2), 5.05-5.42 (4H, m, OCH_2CHC**H$_2$** and CHCHC**H$_2$**), 4.95-5.00 (1H, m, C**H**CO_2CH_3), 4.49-4.51 (2H, m, OC**H$_2$**$CHCH_2$), 4.25-4.38 (2H, m, C**H**CH_2Ph and C**H**$CH(CH_3)_2$), 3.74 (3H, s, $CHCO_2C$**H$_3$**), 3.01-3.05 (2H, m, CHC**H$_2$**Ph), 2.04-2.11 (1H, m, CHC**H**$(CH_3)_2$), 1.45 (9H, s, $C(C$**H$_3$**$)_3$), 0.89 (3H, d J=7.1Hz, $CHCH(C$**H$_3$**$)_2$), 0.87 (3H, d J=7.1Hz, CHC**H**$(CH_3)_2$)

HRMS (ES) 518.2878 (MH^+). $C_{27}H_{39}N_3O_7$ requires 518.2866

(*E/Z*)-(6S,9S,12S)-12-tertButoxycarbonylamino-9-isopropyl-8,11-dioxo-2-oxa-7,10-diazabicyclo-[12.2.2]octadeca-1(17),4,14(18),15-tetraene-6-carboxylic acid methyl ester (4.29)



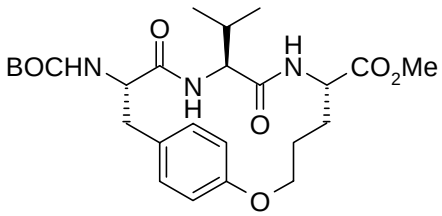
Diene **4.28** (0.830g, 1.60 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.251g, 32%. A 1:3.8 ratio of geometric isomers was obtained. R_f = 0.32 and 0.35 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR for major isomer from mixture (500 MHz in $CDCl_3$) 6.95 (2H, d J=8.1Hz, Ar-**H**), 6.78 (2H, d J=8.1Hz, Ar-**H**), 5.84 (1H, d J=8.0Hz, N**H** Tyr), 5.38-5.47 (2H, m, $OCH_2C\mathbf{H}CHCH$ and $OCH_2CHC\mathbf{H}CH$), 4.91-4.94 (1H, m, $C\mathbf{H}CO_2CH_3$), 4.61-4.73 (2H, m, $OC\mathbf{H_2}CHCHCH$), 4.04-4.13 (2H, m, $C\mathbf{H}CH_2Ph$ and $C\mathbf{H}CH(CH_3)_2$), 3.78 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.03 (1H, dd J=5.1Hz, J=12.5Hz, $CHC\mathbf{H_2}Ph$), 2.68 (1H, dd J=12.5Hz, J=12.5Hz, $CHC\mathbf{H_2}Ph$), 1.92-1.97 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.45 (9H, s, $C(C\mathbf{H_3})_3$), 0.81-0.85 (6H, m, $CHCH(C\mathbf{H_3})_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 7.19 (2H, d J=8.3Hz, Ar-**H**), 4.22-4.30 (2H, m, $C\mathbf{H}CH_2Ph$ and $C\mathbf{H}CH(CH_3)_2$), 3.74 (3H, s, $CHCO_2C\mathbf{H_3}$)

HRMS (ES) 490.2546 (MH^+). $C_{25}H_{35}N_3O_7$ requires 490.2553

(6S,9S,12S)-12-tert-Butoxycarbonylamino-9-isopropyl-8,11-dioxo-2-oxa-7,10-diazabicyclo-[12.2.2]-octadeca-1(17),14(18),15-triene-6-carboxylic acid methyl ester (4.30)

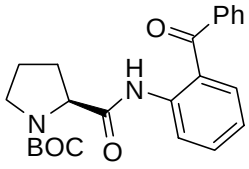


The unsaturated macrocycle **4.29** (0.251g, 0.513 mmol) was hydrogenated in 20 mL of methanol using General Procedure G1 to afford a brown solid, 0.131g, 52%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 6.96 (2H, d J=8.2Hz, Ar-**H**), 6.71 (2H, d J=8.2Hz, Ar-**H**), 6.15 (1H, d J=7.1Hz, N**H** Val), 5.93 (1H, d J=8.2Hz, N**H** Gly), 5.28 (1H, d J=8.6Hz, N**H** Tyr), 4.53-4.55 (1H, m, $C\mathbf{H}CO_2CH_3$), 4.23-4.31 (1H, m, $OC\mathbf{H_2}CH_2CH_2CH$) 4.05-4.22 (2H, m, $C\mathbf{H}CH_2Ph$ and $OC\mathbf{H_2}CH_2CH_2CH$), 3.82-3.84 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.76 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.12 (1H, dd J=5.2Hz, J=12.5Hz, $CHC\mathbf{H_2}Ph$), 2.62 (1H, dd J=12.5Hz, J=12.5Hz, $CHC\mathbf{H_2}Ph$), 2.02-2.08 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.86-1.92 (1H, m, $OCH_2CH_2C\mathbf{H_2}CH$),

1.80 (1H, m, $OCH_2C\mathbf{H_2}CH_2CH$), 1.49-1.57 (2H, m, $OCH_2CH_2C\mathbf{H_2}CH$ and $OCH_2C\mathbf{H_2}CH_2CH$), 1.47 (9H, s, $C(C\mathbf{H_3})_3$), 0.89 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$), 0.86 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$)
HRMS (ES) 492.2700 (MH^+). $C_{25}H_{37}N_3O_7$ requires 492.2710

(S)-2-(2-Benzoylphenylcarbamoyl)pyrrolidine-1-carboxylic acid tertbutyl ester (4.31) Lit[5]

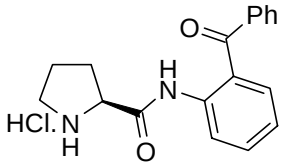


N-BOC-Pro-H (5.00g, 23.2 mmol) was reacted with 2-aminobenzophenone using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 6.30g, 69%. R_f = 0.38 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) (Compound exists as a mixture of rotamers) 11.35 and 11.28 (1H, bs, N**H**), 8.66-8.72 and 8.26-8.28 (1H, bm, Ar-**H**), 7.69 (2H, d J=7.1Hz, Ar-**H**), 7.55-7.58 (3H, m, Ar-**H**), 7.47 (2H, dd J=6.9Hz, J=6.9Hz, Ar-**H**), 7.06-7.13 (1H, m, Ar-**H**), 4.42-4.49 and 4.29 (1H, m and dd J=3.6Hz, J=6.1Hz, C**H**$CH_2CH_2CH_2N$), 3.71-3.80 (1H, bm, $CHCH_2CH_2C\mathbf{H_2}N$), 3.60 and 3.44-3.50 (1H, dd and m, J=1.8Hz, J=6.0Hz, $CHCH_2CH_2C\mathbf{H_2}N$), 3.09 and 2.55 (1H, dd and dd J=7.0Hz, J=12.4Hz and J=6.3Hz, J=13.7Hz, $CHC\mathbf{H_2}CH_2CH_2N$), 2.26-2.34 and 2.20-2.22 (1H, m and m, $CHC\mathbf{H_2}CH_2CH_2NH$), 2.12-2.18 and 1.91 (2H, m and ddd J=6.6Hz, J=6.6Hz, J=11.7Hz, $CHCH_2C\mathbf{H_2}CH_2N$) 1.42 and 1.30 (9H, s and s, $C(C\mathbf{H_3})_3$)

LRMS (ES) 395.2 (MH^+). $C_{23}H_{26}N_2O_4$ requires 395.2

(S)-Pyrrolidine-2-carboxylic acid (2-benzoylphenyl)amide hydrochloride (4.32) Lit[5]

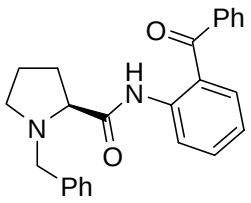


N-BOC protected compound **4.31** (6.30g, 16.0 mmol) was reacted using General Procedure I2. This was partitioned between EtOAc and 1M $NaOH_{(aq)}$. The aqueous phase was extracted twice more with EtOAc. To the combined organic extracts 2M HCl in diethyl ether was added (20 mL). This was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a yellow solid, 5.28g, 100%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 11.61 (1H, bs, N**H**Ph), 8.44 (1H, d J=8.7Hz, Ar-**H**), 7.72 (2H, dd J=0.8Hz, J=7.4Hz, Ar-**H**), 7.58 (1H, dd J=7.4Hz, J=7.4Hz, Ar-**H**), 7.45-7.52 (4H, m, Ar-**H**), 7.10 (1H, dd J=7.4Hz, J=7.4Hz, Ar-**H**), 5.54 (1H, bs, $CHCH_2CH_2CH_2N\mathbf{H}$), 4.18 (1H, dd J=5.8Hz, J=8.7Hz, C**H**$CH_2CH_2CH_2NH$), 3.13-3.22 (2H, m, $CHCH_2CH_2C\mathbf{H_2}NH$), 2.28 (1H, dddd J=8.0Hz, J=8.0Hz, J=8.7Hz, J=16.0Hz, $CHC\mathbf{H_2}CH_2CH_2NH$), 1.95 (1H, dddd J=5.8Hz, J=7.5Hz, J=7.5Hz, J=16.0Hz, $CHC\mathbf{H_2}CH_2CH_2NH$), 1.77-1.86 (2H, m, $CHCH_2C\mathbf{H_2}CH_2NH$)

LRMS (ES) 295.1 (MH^+). $C_{18}H_{18}N_2O_2$ requires 295.1

(S)-1-Benzyl-pyrrolidine-2-carboxylic acid (2-benzoylphenyl)amide (4.33) Lit[5]



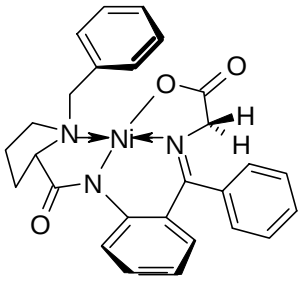
Amine **4.32** (4.90g, 16.6 mmol) was dissolved in anhydrous THF (25 mL) under an atmosphere of argon. This was cooled in ice. Triethylamine (5.57 mL, 2.4 equiv) and benzyl bromide (4.75 mL, 2.4 equiv) were added. This was stirred in ice for one h and then at rt for eighteen h before being concentrated *in vacuo*. The residue was partitioned between EtOAc and water. The organic phase was washed with water and then brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 4.10g, 72%. R_f = 0.32 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 11.53 (1H, bs, N**H**), 8.57 (1H, d J=8.4Hz, Ar-**H**), 7.78 (2H, d J=8.4Hz, Ar-**H**), 7.60 (1H, dd J=7.5Hz, J=7.5Hz, Ar-**H**), 7.48-7.55 (4H, m, Ar-**H**), 7.37 (2H, dd J=2.7Hz, J=6.2Hz, Ar-**H**), 7.07-7.14 (4H, m, Ar-**H**), 3.92 (1H, d J=12.9Hz, NC**H$_2$**Ph), 3.59 (1H, d J=12.9Hz, NC**H$_2$**Ph), 3.31 (1H, dd J=4.8Hz, J=10.1Hz, C**H**$CH_2CH_2CH_2NH$), 3.21 (1H, ddd J=2.1Hz, J=6.7Hz, J=9.7Hz, $CHCH_2CH_2C$**H$_2$**NH), 2.40 (1H, ddd J=6.8Hz, J=9.6Hz, J=9.7Hz, $CHCH_2CH_2C$**H$_2$**NH), 2.21-2.29 (1H, m, CHC**H$_2$**CH_2CH_2NH), 1.96 (1H, ddd J=4.8Hz, J=8.0Hz, J=12.7Hz, CHC**H$_2$**CH_2CH_2NH), 1.69-1.86 (2H, m, $CHCH_2C$**H$_2$**CH_2NH)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 198.0, 174.6, 139.1, 138.5, 138.1, 133.3, 132.5, 130.1, 129.1, 128.3, 128.1, 127.0, 125.3, 122.2, 121.5, 68.2, 59.8, 53.8, 31.0, 24.1

LRMS (ES) 384.9 (MH^+). $C_{25}H_{24}N_2O_2$ requires 385.2

Glycine-nickel-(II)-(N-benzylprolyl)amino]benzophenone (4.34) Lit[5]

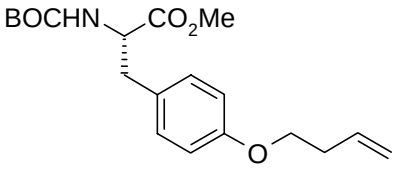


Benzyl proline benzophenone **4.33** (4.30g, 11.2 mmol), glycine (4.20g, 5 equiv) and nickel nitrate hexahydrate (6.50g, 2 equiv) were suspended in methanol (60 mL). The mixture was heated to 60^{o}C and a solution of potassium hydroxide (4.39g, 7 equiv) in water (50 mL) was added and heating continued at 60^{o}C for one h. After cooling the mixture was neutralised to pH 7 using glacial acetic acid, more water (150 mL) was added and

stirred continued at rt for a further eighteen h The red precipitate was collected using suction filteration. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 4.10g, 72%. R_f = 0.32 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.29 (1H, dd, J=3.8Hz, J=8.5, Ar-**H**), 8.07 (2H, dd, J=3.8Hz, J=6.3Hz, Ar-**H**), 7.48-7.56 (3H, m, Ar-**H**), 7.43 (2H, dd J=5.5Hz, J=9.3Hz, Ar-**H)**, 7.31 (1H, dd J= 6.7, J=11.6Hz, Ar-**H**), 7.25-7.27 (1H, m, Ar-**H**), 7.18-7.24 (1H, m, Ar-**H**), 7.08-7.13 (1H, m, Ar-**H**), 6.95-7.02 (1H, m, Ar-**H**), 6.80 (1H, dd J=3.8Hz, J=8.5Hz, Ar-**H**), 6.70 (1H, dd J=6.7Hz, J=11.6Hz, Ar-**H**), 4.49 (1H, d J=12.7Hz, NC**H$_2$**Ph), 3.78 (1H, d J=3.7Hz,NC**H$_2$**C(O)), 3.64-3.73 (3H, m, C**H**$CH_2CH_2CH_2$N and NC**H$_2$**C(O) and NC**H$_2$**Ph), 3.47 (1H, ddd, J= 4.6Hz, J=4.6Hz, J=10.6Hz, CHC**H$_2$**CH_2CH_2N), 3.28-3.41 (1H, m, CHC**H$_2$**CH_2CH_2N), 2.52-2.63 (1H, m, $CHCH_2$C**H$_2$**CH_2N), 2.36-2.49 (1H, m, $CHCH_2$C**H$_2$**CH_2N), 2.02-2.20 (2H, m, $CHCH_2CH_2$C**H$_2$**N)

(S)-3-(4-But-3-enyloxyphenyl)-2-tertbutoxycarbonylaminopropionic acid methyl ester (4.36)

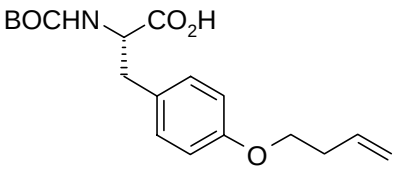


N-BOC-Tyr-OMe (10.0g, 33.9 mmol) was dissolved in DMF (40 mL). To this potassium carbonate (5.62g, 1.2 equiv) and 4-bromo-1-butene (4.13 mL, 1.2 equiv) were added. The mixture was stirred at rt for eighteen h before being diluted with EtOAc (120 mL) and partitioned with 1M $HCl_{(aq)}$. The organic phase was washed with 1M $HCl_{(aq)}$ and then with brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 3.20g, 27%. R_f = 0.82 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.01 (2H, d J=6.3Hz, Ar-**H**), 6.82 (2H, d, J=6.3Hz, Ar-**H**), 5.85-5.94 (1H, m, OCH_2CH_2C**H**CH_2), 5.06-5.31 (2H, m, OCH_2CH_2CHC**H$_2$**), 4.95 (1H, d J=6.1Hz, N**H**), 4.48-4.58 (1H, m, C**H**CO_2CH_3), 3.98 (2H, t, J=6.4Hz, J=6.7Hz, J=6.7Hz, OC**H$_2$**CH_2CHCH_2), 3.70 (3H, s, $CHCO_2$C**H$_3$**), 3.04 (1H, dd J=6.0Hz, J=13.9Hz, CHC**H$_2$**Ph), 2.98 (1H, dd J=5.0Hz, J=13.9Hz, CHC**H$_2$**Ph), 2.53 (2H, dt, J=3.2Hz, J=6.4Hz, J=8.2Hz, OCH_2C**H$_2$**$CHCH_2$), 1.41 (9H, s, C(C**H$_3$**)$_3$)

HRMS (ES) 350.1975 (MH^+). $C_{19}H_{27}NO_5$ requires 350.1967

(S)-3-(4-But-3-enyloxyphenyl)-2-tertbutoxycarbonylamino-propionic acid (4.37)

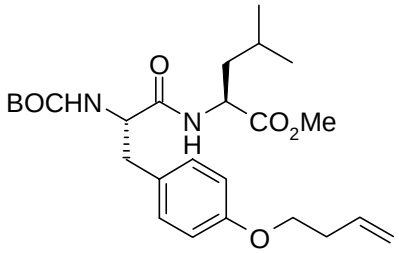


Methyl ester **4.36** (3.20g, 9.16 mmol) was dissolved in THF (30 mL) and hydrolysed using General procedure C1. This afforded a white solid, 2.60g, 85%

^{1}H NMR ppm (500 MHz in CD_3OD) 7.11 (2H, d J=7.3Hz, Ar-**H**), 6.78 (2H, d J=7.3Hz, Ar-**H**), 5.91 (1H, ddt J=2.0Hz, J=6.7Hz, J=17.0Hz, $OCH_2CH_2C\mathbf{H}CH_2$), 5.05-5.16 (2H, m, $OCH_2CH_2CHC\mathbf{H_2}$), 4.16-4.19 (1H, m, $C\mathbf{H}CO_2H$), 3.97 (2H, t J=6.3Hz, $OC\mathbf{H_2}CH_2CHCH_2$), 3.09 (1H, dd J=3.9Hz, J=13.7Hz, $CHC\mathbf{H_2}Ph$), 2.84 (1H, dd J=7.6Hz, J=13.9Hz, $CHC\mathbf{H_2}Ph$), 2.48-2.51 (2H, m, $OCH_2C\mathbf{H_2}CHCH_2$), 1.38 (9H, s, $C(C\mathbf{H_3})_3$)

LRMS (ES) 358.2 (MNa^+). $C_{18}H_{25}NO_5Na$ requires 358.2

(S)-2-[(S)-3-(4-But-3-enyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-4-ethyl-pentanoic acid methyl ester (4.38)



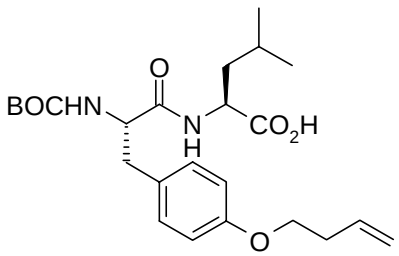
Carboxylic acid **4.37** (2.60g, 7.75 mmol) was reacted with Leu-OMe using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 2.30g, 64%. R_f = 0.34 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.11 (2H, d J=8.5Hz, Ar-**H**), 6.82 (2H, d J=8.5Hz, Ar-**H**), 6.24 (1H, d J=7.5Hz, N**H** Leu), 5.85-5.94 (1H, m, $OCH_2CH_2C\mathbf{H}CH_2$), 5.08-5.17 (2H, m, $OCH_2CH_2CHC\mathbf{H_2}$), 5.01 (1H, bs, N**H** Tyr), 4.54-4.58 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 4.27-4.32 (1H, m, $C\mathbf{H}CH_2Ph$), 3.98 (2H, dt J=2.3Hz, J=6.7Hz, $OC\mathbf{H_2}CH_2CHCH_2$), 3.69 (3H, s, $CO_2C\mathbf{H_3}$), 2.95-3.05 (2H, m, $CHC\mathbf{H_2}Ph$), 2.51-2.55 (2H, m, $OCH_2C\mathbf{H_2}CHCH_2$), 1.53-1.60 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 1.44-1.49 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 1.42 (9H, s, $C(C\mathbf{H_3})_3$), 0.88-0.91 (6H, m, $CHCH_2CH(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 172.8, 171.0, 157.8, 134.4, 130.3, 130.2, 128.4, 116.9, 114.6, 67.1, 55.7, 52.2, 50.6, 41.5, 37.1, 33.6, 28.2, 24.6, 22.7, 21.8

HRMS (ES) 463.2809 (MH^+). $C_{25}H_{38}N_2O_6$ requires 463.2808

(S)-2-[(S)-3-(4-But-3-enyloxy-phenyl)-2-tertbutoxycarbonylaminopropionylamino]-4-methylpentanoic acid (4.39)



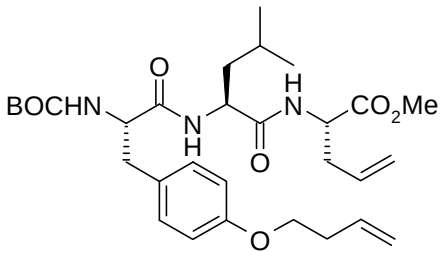
Dipeptide **4.38** (2.30g, 4.97 mmol) was dissolved in THF (30 mL) and hydrolysed using General procedure C1. This afforded a white solid, 2.14g, 96%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.41 (1H, bs, CO_2**H**), 7.09 (2H, d J=8.1Hz, Ar-**H**), 6.80 (2H, d J=8.1Hz, Ar-**H**), 6.70 (1H, d J=7.7Hz, N**H** Leu), 5.84-5.92 (1H, m, $OCH_2CH_2CHCH_2$), 5.29 (1H, bs, N**H** Tyr), 5.07-5.16 (2H, m, $OCH_2CH_2CHCH_2$), 4.54-4.57 (1H, m, $CHCH_2CH(CH_3)_2$), 4.36-4.37 (1H, m, $CHCH_2Ph$), 3.95 (2H, t J=6.5Hz, J=6.5Hz, $OCH_2CH_2CHCH_2$), 2.93-3.02 (2H, m, $CHCH_2Ph$), 2.49-2.51 (2H, m, $OCH_2CH_2CHCH_2$) 1.50-1.69 (3H, m, $CHCH_2CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.38 (9H, s, $C(CH_3)_3$), 0.90-0.92 (6H, m, $CHCH_2CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 175.9, 171.6, 157.8, 134.4, 130.3, 130.2, 128.4, 116.9, 114.6, 67.1, 55.6, 50.8, 41.1, 37.7, 37.1, 30.6, 28.2, 24.6, 22.8, 21.8

LRMS (ES) 449.6 (MH^+). $C_{24}H_{36}N_2O_6$ requires 449.3

(S)-2-{(S)-2-[(S)-3-(4-But-3-enyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-4-methylpentan-oylamino}-pent-4-enoic acid methyl ester (4.40)



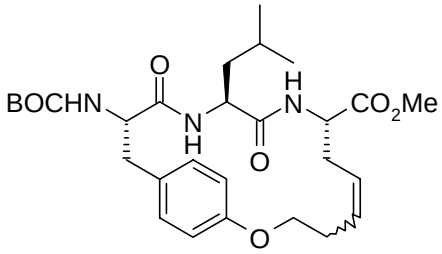
Carboxylic acid **4.39** (2.14g, 4.77 mmol) was reacted with amine **4.14** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 2.24g, 84%. R_f = 0.32 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.09 (2H, d J=8.4Hz, Ar-**H**), 6.93 (1H, d J=7.7Hz, N**H** Gly), 6.80 (2H, d J=8.4Hz, Ar-**H**), 6.71 (1H, d J=7.3Hz, N**H** Leu), 5.89 (1H, tdd J=6.7Hz, J=10.1Hz, J=17.0Hz, $OCH_2CH_2CHCH_2$), 5.68-5.72 (1H, m, $CHCH_2CHCH_2$), 5.09-5.22 (4H, m, $OCH_2CH_2CHCH_2$ and $CHCH_2CHCH_2$), 4.54-4.60 (1H, m, $CHCO_2CH_3$), 4.50 (1H, ddd J=6.0Hz, J=6.0Hz, J=7.3Hz, $CHCH_2CH(CH_3)_2$), 4.36-4.38 (1H, m, $CHCH_2Ph$), 3.96 (2H, t J=6.7Hz, $OCH_2CH_2CHCH_2$), 3.73 (3H, s, $CHCO_2CH_3$), 2.99 (1H, dd J=6.0Hz, J=14.0Hz, $CHCH_2Ph$), 2.93 (1H, dd J=6.9Hz, J=14.0Hz, $CHCH_2Ph$), 2.45-2.58 (4H, m, $CHCH_2CHCH_2$ and $OCH_2CH_2CHCH_2$, 1.54-1.66 (2H, m, $CHCH_2CH(CH_3)_2$), 1.43-1.48 (1H, m, $CHCH_2CH(CH_3)_2$), 1.39 (9H, s, $C(CH_3)_3$), 0.86 (3H, d J=6.3Hz, $CHCH_2CH(CH_3)_2$), 0.86 (3H, d J=6.3Hz, $CHCH_2CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.8, 171.6, 171.5, 157.8, 134.4, 132.2, 130.3, 130.2, 128.6, 118.9, 116.9, 114.5, 79.8, 67.0, 55.6, 52.2, 51.8, 51.5, 41.1, 37.2, 36.3, 36.2, 33.6, 28.2, 24.4, 22.8, 22.2.

HRMS (ES) 560.3346 (MH^+). $C_{30}H_{45}N_3O_7$ requires 560.3336

(*E/Z*)-(8S,11S,14S)-14-tertButoxycarbonylamino-11-isobutyl-10,13-dioxo-2-oxa-9,12-diazabicyclo-[14.2.2]icosa-1(19),5,16(20),17-tetraene-8-carboxylic acid methyl ester (4.41)



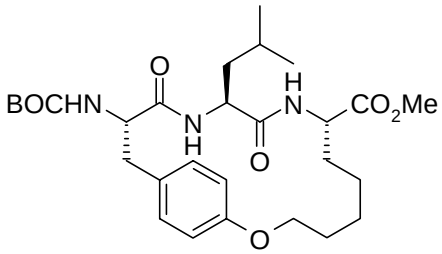
Diene **4.40** (2.20g, 3.57 mmol) was reacted with Grubbs second generation catalyst using General Procedure E2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 2.09g, 100%. A 1:1.9 ratio of geometric isomers was obtained. R_f = 0.42 and 0.43 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR for major isomer from mixture (500 MHz in $CDCl_3$) 7.09 (2H, d J=8.1Hz, Ar-**H**), 6.75 (2H, d J=8.1Hz, Ar-**H**), 6.02 (1H, d J=7.8 Hz, N**H** Leu), 5.96 (1H, d J=8.0Hz, N**H** Gly), 5.40-5.65 (2H, m, $OCH_2C\mathbf{H}CHCH_2CH_2$ and $OCH_2CHC\mathbf{H}CH_2CH_2$), 5.24 (1H, d J=8.3Hz, N**H** Tyr), 4.93 (1H, ddd J=1.2Hz, J=6.2Hz, J=13.3Hz, $OC\mathbf{H_2}CHCHCH_2CH_2$), 4.58-4.77 (1H, m, $OC\mathbf{H_2}CHCHCH_2CH_2$), 4.45 (1H, ddd J=3.4Hz, J=8.6Hz, J=8.8Hz, $C\mathbf{H}CO_2CH_3$), 4.10-4.32 (2H, m, $C\mathbf{H}CH_2Ph$, $C\mathbf{H}CH_2CH(CH_3)_2$), 3.74 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.01 (1H, dd J=4.6Hz, J=12.8Hz, $CHC\mathbf{H_2}Ph$), 2.82-2.86 (1H, m, $CHC\mathbf{H_2}Ph$), 2.37-2.48 (2H, m, $OCH_2CHCHC\mathbf{H_2}CH_2$), 2.20-2.36 (2H, m, $OCH_2CHCHCH_2C\mathbf{H_2}$), 1.47-1.60 (3H, m, $CHC\mathbf{H_2}CH(CH_3)_2$ and $CHCH_2C\mathbf{H}(CH_3)_2$), 1.45 (9H, s, $C(C\mathbf{H_3})_3$), 0.84-0.90 (6H, m, $CHC\mathbf{H_2}CH(CH_3)_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 7.12 (2H, d J=8.4Hz, Ar-**H**), 6.79 (2H, d J=8.4Hz, Ar-**H**), 6.20 (1H, d J=7.4Hz, N**H** Leu), 6.15 (1H, d J=8.6Hz, N**H** Gly), 5.73 (1H, d J=7.6Hz, N**H** Tyr)

HRMS (ES) 532.3034 (MH^+). $C_{28}H_{41}N_3O_7$ requires 532.3023

(8S,11S,14S)-14-tertButoxycarbonylamino-11-isobutyl-10,13-dioxo-2-oxa-9,12-diazabicyclo-[14.2.2]icosa-1(19),16(20),17-triene-8-carboxylic acid methyl ester (4.42)



The unsaturated macrocycle **4.41** (2.30g, 4.33 mmol) was hydrogenated in 50 mL of methanol using General Procedure G3 to afford a brown solid, 0.600g, 26%

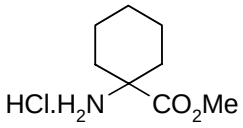
^{1}H NMR ppm (500 MHz in CD_3OD) 8.13 (1H, d J=7.6Hz, N**H** Leu), 7.44 (1H d J=8.3Hz, N**H** Gly), 7.04 (2H, d J=8.5Hz, Ar-**H**), 6.73 (2H, d J=8.5Hz, Ar-**H**), 4.20-4.27 (2H, m, C**H**CO_2CH_3 and C**H**$CH_2CH(CH_3)_2$), 4.11-4.20 (2H, m, O**CH_2**$CH_2CH_2CH_2CH_2$ and C**H**CH_2Ph), 3.94-3.99 (1H, m, O**CH_2**$CH_2CH_2CH_2CH_2$), 3.65 (3H, s, $CHCO_2C$**H_3**), 2.93 (1H, dd J=4.9Hz, J=12.6Hz, CHC**H_2**Ph), 2.71 (1H, dd J=11.8Hz, J=12.6Hz, CHC**H_2**Ph), 1.63-1.74 (3H, m, OCH_2C**H_2**$CH_2CH_2CH_2$ and $OCH_2CH_2CH_2CH_2C$**H_2**), 1.51-1.60 (3H, m, CHC**H_2**$CH(CH_3)_2$ and $CHCH_2C$**H**$(CH_3)_2$), 1.43 (9H, s, $C(C$**H_3**$)_3$), 1.21-1.37 (5H, m, OCH_2CH_2C**H_2**CH_2CH_2 and $OCH_2CH_2CH_2C$**H_2**CH_2 and $OCH_2CH_2CH_2CH_2C$**H_2**), 0.86 (6H, d J=6.4Hz, $CHCH_2CH(C$**H_3**$)_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 172.7, 172.1, 171.4, 157.4, 156.0, 130.0, 128.1, 114.8, 79.2, 66.3, 56.3, 51.4, 51.3, 42.6, 37.0, 31.2, 27.4, 27.3, 24.1, 23.9, 22.1, 21.6

HRMS (ES) 534.3107 (MH^+). $C_{28}H_{43}N_3O_7$ requires 534.3179

FTIR (KBr) 3302, 2929, 1944, 1747, 1741, 1685, 1647, 1541, 1508, 1248

1-Aminocyclohexanecarboxylic acid methyl ester hydrochloride (4.44) Lit[5]

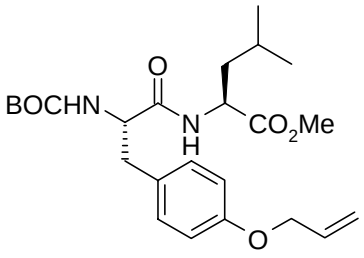


1-Amino-cyclohexanecarboxylic acid **4.43** (5.00g, 34.9 mmol) was suspended in methanol (100 mL). This was esterified using General procedure F1 to afford a white solid. 6.76g, 100%.

^{1}H NMR ppm (500 MHz in CD_3OD) 3.74 (3H, s, CO_2C**H_3**), 1.97-2.03 (2H, m, CH_2CH_2C**H_2**CH_2CH_2), 1.61-1.71 (4H, m, C**H_2**$CH_2CH_2CH_2C$**H_2**), 1.41-1.56 (4H, m, CH_2C**H_2**CH_2C**H_2**CH_2)

LRMS (ES) 158.1 (MH^+). $C_8H_{15}NO_2$ requires 158.1

(S)-2-[(S)-3-(4-Allyloxy-phenyl)-2-tertbutoxycarbonylaminopropionylamino]-4-methyl-pentanoic acid methyl ester (4.45)



N-BOC-O-allyl-Tyr-H (19.7g, 64.1 mmol) was reacted with Leu-OMe using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 23.7g, 80%. R_f = 0.31 (1/2 (EtOAc / (50/70) Pet ether)).

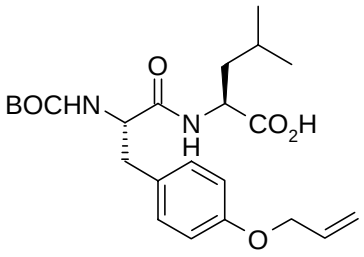
^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.08 (2H, d J=8.5Hz, Ar-**H**), 6.79 (2H, d, J=8.5Hz, Ar-**H**), 6.43 (1H, d, J=7.6Hz, N**H** Leu), 6.04 (1H, tdd J=5.3Hz, J=5.3Hz, J=10.5, J=17.1Hz, CH_2C**H**CH_2), 5.26-5.42 (2H, m,

$CH_2CHC\mathbf{H_2}$), 5.09 (1H, d, J=6.6Hz, N**H** Tyr), 4.54-4.57 (1H, m, C**H** Leu), 4.47 (2H, d, J=5.3Hz, $C\mathbf{H_2}CHCH_2$), 4.30-4.36 (1H, m, $C\mathbf{H}CH_2Ph$), 3.66 (3H, s, $CO_2C\mathbf{H_3}$), 2.88-3.02 (2H, m, $CHC\mathbf{H_2}Ph$), 1.54-1.62 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 1.43-1.51 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$) 1.38 (9H, s, $(C\mathbf{H_3})_3$), 0.91 (3H, d J=6.6 Hz, $CHCH_2(C\mathbf{H_3})_2$), 0.89 (3H, d J=6.6 Hz, $CHCH_2(C\mathbf{H_3})_2$).

^{13}C NMR ppm (75 MHz $CDCl_3$). 174.1, 172.8, 171.4, 157.5, 155.5, 133.2, 130.4, 130.3, 128.7, 117.4, 114.7, 68.7, 54.3, 52.1, 50.7, 41.3, 37.3, 28.2, 24.6, 22.7, 21.8

HRMS (ES) 449.2662 (MH^+). $C_{24}H_{36}N_2O_6$ requires 449.2651

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-4-methylpentanoic acid (4.46)

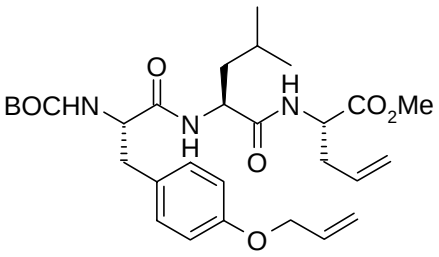


Dipeptide **4.45** (23.6g, 52.6 mmol) was dissolved in THF (100 mL) and hydrolysed using General procedure C1. This afforded a white solid, 22.1g, 97%

^1H-NMR (500 MHz in $CDCl_3$) 7.10 (2H, d, J=8.5Hz, Ar-**H**), 6.82 (2H, d J=8.5Hz, Ar-**H**), 6.58 (1H, d J=7.9Hz, N**H** Leu), 6.03 (1H, tdd J=5.3Hz, J=5.3Hz, J=10.5Hz, J=17.3Hz, $CH_2C\mathbf{H}CH_2$), 5.25-5.42 (2H, m, $CH_2CHC\mathbf{H_2}$), 5.22 (1H, bs, N**H** Tyr), 4.54-4.58 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 4.48 (2H, d, J=5.2Hz, $C\mathbf{H_2}CHCH_2$), 4.34-4.40 (1H, m, $C\mathbf{H}CH_2Ph$), 2.96-3.02 (2H, m, $CHC\mathbf{H_2}Ph$), 1.58-1.70 (2H, m, $CHCHC\mathbf{H_2}(CH_3)_2$), 1.50-1.56 (1H, m, $CHC\mathbf{H}CH_2(CH_3)_2$), 1.39 (9H, s, $C(C\mathbf{H_3})_3$), 0.92 (3H, d J=6.2Hz, $CHCHCH_2(C\mathbf{H_3})_2$), 0.91 (3H, d J=6.2 Hz, $CHCHCH_2(C\mathbf{H_3})_2$)

LRMS (ES) 435.2 (MH^+). $C_{23}H_{34}N_2O_6$ requires 435.2

(S)-2-{(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-4-methyl pentanoylamino}-pent-4-enoic acid methyl ester (4.47)



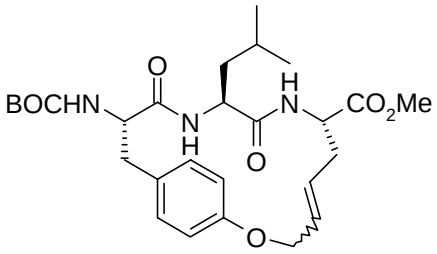
Carboxylic acid **4.46** (10.0g, 23.0 mmol) was reacted with amine **4.14** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 12.2g, 97%. R_f = 0.46 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR (500 MHz in $CDCl_3$) 7.09 (2H, d J=8.5Hz, Ar-**H**), 6.82 (2H, d J=8.5Hz, Ar-**H**), 6.65 (1H, d J=7.6Hz, N**H** Gly), 6.47 (1H, d J=8.1Hz, N**H** Leu), 6.04 (1H, tdd J= 5.3Hz, J=5.3Hz, J=10.5Hz, J=17.0Hz, $OCH_2C\mathbf{H}CH_2$), 5.62-5.71 (1H, m, $CHCH_2C\mathbf{H}CH_2$), 5.08-5.44 (4H, m, $OCH_2CHC\mathbf{H_2}$ and $CHCH_2CHC\mathbf{H_2}$), 4.99 (1H, d J=7.3Hz, N**H** Tyr), 4.57-4.63 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 4.49 (2H, d J=5.2Hz, $OC\mathbf{H_2}CHCH_2$), 4.41-4.47 (1H, m, $C\mathbf{H}CH_2CHCH_2$), 4.25-4.34 (1H, m, $C\mathbf{H}CH_2Ph$), 3.73 (3H, s, $CO_2C\mathbf{H_3}$), 2.96-3.05 (2H, m, $CHC\mathbf{H_2}Ph$), 2.45-2.61 (2H, m, $CHC\mathbf{H_2}CHCH_2$), 1.52-1.68 (2H, m, $CHCHC\mathbf{H_2}(CH_3)_2$) 1.43-1.50 (1H, m, $CHC\mathbf{H}CH_2(CH_3)_2$) 1.39 (9H, s, $C(C\mathbf{H_3})_3$), 0.90 (3H, d J=6.4Hz, $CHCHCH_2(C\mathbf{H_3})_2$), 0.89 (3H, d J=6.4Hz, $CHCHCH_2(C\mathbf{H_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 171.8, 171.5, 171.3, 157.5, 155.4, 133.2, 132.2, 132.0, 130.3, 130.1, 128.5, 119.2, 119.0, 117.6, 114.8, 80.2, 68.7, 55.6, 52.3, 51.7, 40.9, 40.7, 37.0, 36.2, 28.2, 24.4, 22.8, 22.0

HRMS (ES) 546.3180 (MH^+). $C_{29}H_{43}N_3O_7$ requires 546.3179

(*E/Z*)-(7S,10S,13S)-13-tertButoxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (4.48)



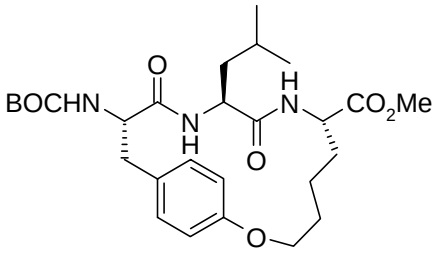
Diene **4.47** (2.00g, 3.67 mmol) was reacted with Grubbs second generation catalyst using General Procedure E2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 1.73g, 91%. A 1:9 ratio of geometric isomers was obtained. R_f = 0.28 and 0.30 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR for major isomer from mixture (500 MHz in $CDCl_3$) 7.01 (2H, d J=5.4Hz, Ar-**H**), 6.78 (2H, d J=5.4Hz, Ar-**H**), 5.87 (1H, d J=8.6Hz, N**H** Gly), 5.81 (1H, d J=7.1Hz, N**H** Leu), 5.40-5.58 (2H, m, $OCH_2C\mathbf{H}CHCH_2$ and $OCH_2CHC\mathbf{H}CH_2$), 5.34 (1H, d, J=8.6Hz, N**H** Tyr), 4.73 (1H, ddd J=3.2Hz, J=8.6Hz, J=9.2Hz, $C\mathbf{H}CO_2CH_3$), 4.55-4.68 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.09-4.20 (2H, m, $C\mathbf{H}CH_2Ph$ and $C\mathbf{H}CH_2CH(CH_3)_2$), 3.74 (3H, s, $CO_2C\mathbf{H_3}$), 3.08 (1H, dd J=4.9Hz, J=12.6Hz, $CHC\mathbf{H_2}Ph$), 2.65-2.74 (1H, m, $CHC\mathbf{H_2}Ph$), 2.26-2.34 (2H, m, $OCH_2CHCHC\mathbf{H_2}$), 1.82-1.90 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 1.52-1.58 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 1.44 (9H, s, $(C\mathbf{H_3})_3$), 0.84-0.88 (6H, m, $CHCH_2CH(C\mathbf{H_3})_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 3.77 (3H, s, $CO_2C\mathbf{H_3}$), 2.48-2.52 (1H, m, $CHC\mathbf{H_2}Ph$), 1.48 (9H, s, $(C\mathbf{H_3})_3$), 0.89-0.94 (6H, m, $CH_2CH(C\mathbf{H_3})_2$)

HRMS (ES) 518.2869 (MH^+). $C_{27}H_{39}N_3O_7$ requires 518.2866

(7S,10S,13S)-13-tertButoxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (4.49)

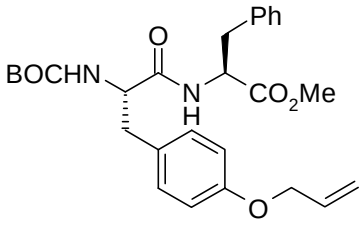


The unsaturated macrocycle **4.48** (6.90g, 13.3 mmol) was hydrogenated in 100 mL of methanol using General Procedure G3 to afford a brown solid, 6.80g, 98%

^{1}H-NMR (500 MHz in $CDCl_3$) 7.05 (2H, d J=7.8Hz, Ar-**H**), 6.79 (2H, d, J=7.8Hz, Ar-**H**), 6.08 (1H, d J=6.1Hz N**H** Gly), 5.74 (1H, d, J=8.2Hz, N**H** Leu), 5.22 (1H, d J=8.8Hz, N**H** Tyr), 4.38-4.42 (1H, m, C**H**CH_2Ph), 4.31-4.36 (1H, m, C**H**CO_2CH_3), 4.12-4.28 (2H, m, O**CH_2**$CH_2CH_2CH_2$), 3.96-4.06 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.73 (3H, s, CO_2C**H_3**), 3.11 (1H, dd J=5.3Hz, J=12.2Hz, CHC**H_2**Ph), 2.65 (1H, dd, J=12.2Hz, J=12.2Hz CHC**H_2**Ph), 1.68-1.74 (2H, m, OCH_2C**H_2**CH_2CH_2), 1.49-1.63 (3H, m, CHC**H_2**$CH(CH_3)_2$ and $CHCH_2$C**H**$(CH_3)_2$), 1.45 (9H, s, C(C**H_3**$)_3$), 1.20-1.40 (2H, m, $OCH_2CH_2CH_2$C**H_2**), 0.82-0.85 (6H, m, $CHCH_2CH$(C**H_3**$)_2$)

HRMS (ES) 520.3031 (MH^+). $C_{27}H_{41}N_3O_7$ requires 520.3023

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylamino-propionylamino]-3-phenylpropionic acid methyl ester (4.50)



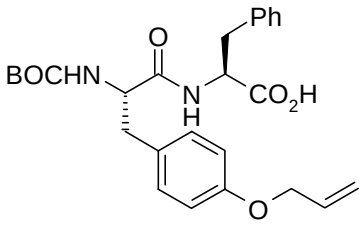
N-BOC-O-allyl-Tyr-H (5.00g, 16.3 mmol) was reacted with Phe-OMe using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 6.36g, 81%. R_f = 0.25 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.21-7.24 (3H, m, Ar-**H** (Phe)), 7.08 (2H, d J=7.7Hz, Ar-**H** (Tyr)), 6.99 (2H, dd J=1.5, J=7.4, Ar-**H** (Phe)), 6.82 (2H, d J=7.7Hz, Ar-**H** (Tyr)), 6.38 (1H, d J=7.1Hz, N**H** Phe), 5.98-6.07 (1H, m, OCH_2C**H**CH_2), 5.25-5.41 (2H, m, OCH_2CHC**H_2**), 5.02 (1H, bs, N**H** Tyr) 4.74-4.81 (1H, m, C**H**CO_2CH_3), 4.48-4.49 (2H, m, O**CH_2**$CHCH_2$), 4.25-4.34 (1H, m, NHC**H**C(O)NH), 3.66 (3H, s, $CHCO_2$C**H_3**), 3.02-3.08 (2H, m, CHC**H_2**Ph (Phe)), 2.94-3.02 (2H, m, CHC**H_2**Ph (Tyr)), 1.40 (9H, s, C(C**H_3**$)_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.3, 170.8, 157.5, 135.6, 133.2, 130.3, 129.1, 128.4, 127.0, 117.5, 114.8, 68.7, 53.2, 52.2, 37.9, 37.3, 28.2

HRMS (ES) 483.2496 (MH^+). $C_{27}H_{34}N_2O_6$ requires 483.2495

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-phenylpropionic acid (4.51)



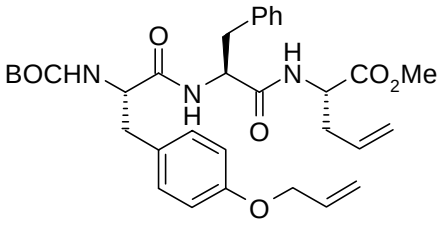
Dipeptide **4.50** (6.30g, 13.1 mmol) was dissolved in THF (30 mL) and hydrolysed using General procedure C1. This afforded a white solid, 5.69g, 93%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.82 (1H, bs, CO_2**H**), 7.19-7.26 (3H, m, Ar-**H** (Phe)), 7.04-7.08 (4H, m, Ar-**H** (Tyr) and Ar-**H** (Phe)), 6.81 (2H, d J=8.2Hz, Ar-**H** (Tyr)), 6.67 (1H, d J=7.7Hz, N**H** Phe), 6.02 (1H, tdd J=5.3Hz, J=10.5Hz, J=17.3Hz, OCH_2C**H**CH_2), 5.24-5.42 (2H, m, OCH_2CHC**H$_2$**), 5.22 (1H, bs, N**H** Tyr), 4.76-4.78 (1H, m, C**H**CO_2CH_3), 4.47 (2H, d J=4.8Hz, OC**H$_2$**$CHCH_2$), 4.37-4.39 (1H, m, NHC**H**C(O)NH), 3.13 (1H, dd J=4.4Hz, J=13.3Hz, CHC**H$_2$**Ph (Phe)), 2.91-3.01 (3H, m, CHC**H$_2$**Ph (Phe) and CHC**H$_2$**Ph (Tyr)), 1.39 (9H, s, C(C**H$_3$**)$_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 176.6, 174.5, 171.5, 135.7, 133.3, 130.4, 130.3, 129.4, 128.5, 127.1, 117.6, 114.8, 68.8, 37.5, 28.3, 20.8

LRMS (ES) 469.2 (MH^+). $C_{26}H_{33}N_2O_6$ requires 469.2

(S)-2-{(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylamino-propionylamino]-3-phenylpropionyl-amino}-pent-4-enoic acid methyl ester (4.52)



Carboxylic acid **4.13** (2.00g, 4.27 mmol) was reacted with amine **4.14** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 1.61g, 65%. R_f = 0.12 (1/2 (EtOAc / (50/70) Pet ether)).

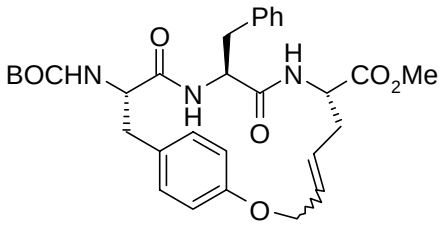
^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.20-7.27 (3H, m, Ar-**H** (Phe)), 7.03-7.12 (4H, m, Ar-**H** (Phe) and Ar-**H** (Tyr)), 6.83 (2H, d J=8.6Hz, Ar-**H** (Tyr)), 6.51 (1H, d J=6.8Hz, N**H** Phe), 6.37 (1H, d J=6.1Hz, N**H** Gly), 6.04

(1H, tdd J=5.3Hz, J=10.6Hz, J=17.4Hz, $OCH_2C\mathbf{H}CH_2$), 5.57 (1H, tdd J=7.2Hz, J=7.2Hz, J=10.3Hz, J=17.3Hz, $CHCH_2C\mathbf{H}CH_2$), 5.26-5.43 (2H, m, $OCH_2CHC\mathbf{H_2}$), 5.00-5.05 (2H, m, $CHCH_2CHC\mathbf{H_2}$), 4.87 (1H, d J=7.1Hz, N**H** Tyr), 4.61-4.64 (1H, m, C**H**CH_2Ph (Phe)), 4.49-4.55 (3H, m, $OC\mathbf{H_2}CHCH_2$ and C**H**CO_2CH_3), 4.26-4.30 (1H, m, C**H**CH_2Ph (Tyr)), 3.70 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.10 (1H, dd J=5.7Hz, J=13.7Hz, CHC**H$_2$**Ph (Phe)), 2.92-2.99 (3H, m, CHC**H$_2$**Ph (Phe) and CHC**H$_2$**Ph (Tyr)), 2.50 (1H, ddd J=5.9Hz, J=6.9Hz, J=13.8Hz, $CHC\mathbf{H_2}CHCH_2$), 2.40 (1H, ddd J=5.9Hz, J=5.9Hz, J=13.8Hz, $CHC\mathbf{H_2}CHCH_2$), 1.37 (9H, s, $C(C\mathbf{H_3})_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.3, 171.1, 170.0, 157.6, 136.2, 133.2, 132.0, 130.2, 129.3, 128.6, 128.3, 127.0, 119.1, 117.6, 114.9, 68.7, 54.1, 52.3, 51.9, 37.9, 36.1, 28.2

HRMS (ES) 580.3027 (MH^+). $C_{32}H_{42}N_3O_7$ requires 580.3023

(*E/Z*)-(7S,10S,13S)-10-Benzyl-13-tertbutoxycarbonylamino-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (4.53)



Diene **4.52** (1.00g, 1.73 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.485g, 51%. A 1:6.25 ratio of geometric isomers was obtained R_f = 0.29 and 0.30 (2/1 (EtOAc / (50/70) Pet ether)).

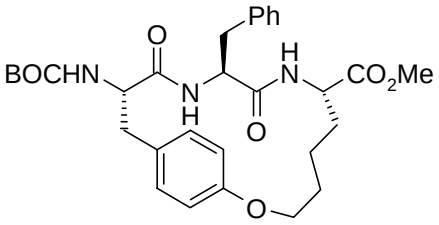
^{1}H-NMR for major isomer from mixture (500 MHz in $CDCl_3$) 7.21-7.28 (3H, m, Ar-**H** (Phe)), 7.03-7.10 (4H, m, Ar-**H** (Phe) and Ar-**H** (Tyr)), 6.70 (2H, d J=8.3Hz, Ar-**H** (Tyr)), 6.18 (1H, d J=8.4Hz, N**H** Gly), 5.85 (1H, d J=6.6Hz, N**H** Phe), 5.58 (1H, d J=8.0Hz, N**H** Tyr), 5.36-5.46 (2H, m, $OCH_2C\mathbf{H}CHCH_2$ and $OCH_2CHC\mathbf{H}CH_2$), 4.68-4.70 (1H, m, C**H**CO_2CH_3), 4.50-4.61 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.42-4.46 (1H, m, C**H**CH_2Ph (Phe)), 4.23-4.27 (1H, m, C**H**CH_2Ph (Tyr)), 3.72 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.09-3.17 (2H, m, CHC**H$_2$**Ph (Phe) and CHC**H$_2$**Ph (Tyr)), 2.86 (1H, dd J=7.0Hz, J=13.4Hz, CHC**H$_2$**Ph (Phe)), 2.59-2.67 (2H, m, CHC**H$_2$**Ph (Tyr) and $OCH_2CHCHC\mathbf{H_2}$), 2.24-2.31 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 1.50 (9H, s, $C(C\mathbf{H_3})_3$)

Selected ^{1}H-NMR for minor isomer from mixture: 3.69 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.32-2.38 (1H, m, $OCH_2CHCHC\mathbf{H_2}$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.6, 170.8, 169.4, 156.0, 155.0, 135.8, 130.3, 129.4, 128.4, 128.1, 127.3, 126.9, 115.7, 79.8, 66.4, 57.0, 54.2, 52.5, 51.5, 39.3, 35.2, 28.4

HRMS (ES) 552.2699 (MH^+). $C_{30}H_{37}N_3O_7$ requires 552.2710

(7S,10S,13S)-10-Benzyl-13-tertbutoxycarbonylamino-9,12-dioxo-2-oxa-8, 11-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (4.54)



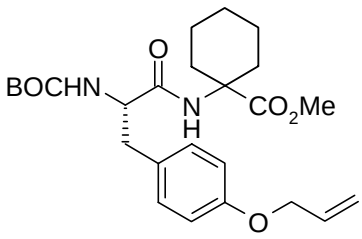
The unsaturated macrocycle **4.53** (0.235g, 0.426 mmol) was hydrogenated in 10 mL of methanol using General Procedure G1 to afford a brown solid, 0.0590g, 25%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.19-7.27 (3H, m, Ar-**H** (Phe)), 7.10 (2H, d J=7.0Hz, Ar-**H** (Phe)), 7.04 (2H, d J=7.6Hz, Ar-**H** (Tyr)), 6.76 (2H, d J=7.6Hz, Ar-**H** (Tyr)), 6.11 (1H, d J=6.5Hz, N**H** Phe), 5.56 (1H, d J=7.6Hz, N**H** Gly), 5.35 (1H, d J=7.8Hz, N**H** Tyr), 4.42 (1H, ddd J=3.6Hz, J=7.6Hz, J=7.6Hz, C**H**CO_2CH_3), 4.16-4.25 (2H, m, C**H**CH_2Ph (Tyr) and O**CH_2**$CH_2CH_2CH_2$), 4.07-4.11 (1H, m, O**CH_2**$CH_2CH_2CH_2$), 4.02 (1H, ddd, J=6.5Hz, J=8.9Hz, J=12.8Hz, C**H**CH_2Ph (Phe)), 3.66 (3H, s, $CHCO_2C$**H_3**), 3.16 (1H, dd J=5.6Hz, J=12.1Hz, CHC**H_2**Ph (Tyr)), 3.07 (1H, dd J=3.9Hz, J=12.8Hz, CHC**H_2**Ph (Phe)), 2.78 (1H, dd J=8.9Hz, J=12.8Hz, CHC**H_2**Ph (Phe)), 2.63 (1H, dd J=12.1Hz, J=12.1Hz, CHC**H_2**Ph (Tyr)), 1.80-1.89 (1H, m, $OCH_2CH_2CH_2C$**H_2**), 1.65-1.73 (1H, m, OCH_2C**H_2**CH_2CH_2), 1.32-1.59 (11H, m, $OCH_2CH_2CH_2C$**H_2** and OCH_2CH_2C**H_2**CH_2 and $C(C$**H_3**$)_3$), 1.24-1.32 (1H, m, OCH_2CH_2C**H_2**CH_2), 1.12-1.20 (1H, m, OCH_2C**H_2**CH_2CH_2)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.3, 170.2, 169.2, 157.4, 136.0, 129.3, 128.4, 126.9, 115.6, 66.6, 57.1, 54.8, 52.4, 51.4, 39.5, 39.3, 31.2, 28.3, 28.1, 20.5

HRMS (ES) 554.2871 (MH^+). $C_{30}H_{39}N_3O_7$ requires 554.2866

1-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]cyclohexanecarboxylic acid methyl ester (4.55)



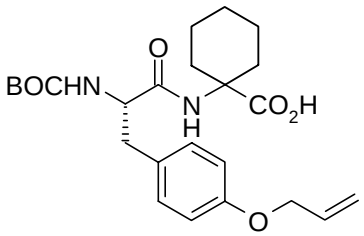
N-BOC-O-allyl-Tyr-H (5.00g, 16.3 mmol) was reacted with amine **4.44** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 4.42g, 59%. R_f = 0.41 (1/2 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.17 (2H, d J=8.4Hz, Ar-**H**), 6.85 (2H, d J=8.4Hz, Ar-**H**), 6.18 (1H, bs, N**H** Cy), 6.04 (1H, tdd J=5.2Hz, J=10.6Hz, J=16.8Hz, OCH_2C**H**CH_2), 5.26-5.43 (2H, m, OCH_2CHC**H_2**), 5.08 (1H, d

J=7.2Hz, N**H** Tyr), 4.51 (1H, d J=5.2Hz, OC**H₂**CHCH₂), 4.28 (1H, ddd J=6.3Hz, J=7.7Hz, J=14.0Hz, C**H**CH₂Ph), 3.68 (3H, s, CO₂C**H₃**), 3.04 (1H, dd, J=6.3Hz, J=14.0Hz, CHC**H₂**Ph), 2.97 (1H, dd J=7.7Hz, J=14.0Hz, CHC**H₂**Ph), 1.90-1.93 (2H, m, C**H₂**CH₂CH₂CH₂C**H₂**), 1.75-1.78 (2H, m, C**H₂**CH₂CH₂CH₂C**H₂**), 1.50-1.56 (3H, m, CH₂C**H₂**C**H₂**C**H₂**CH₂), 1.42 (9H s, C(C**H₃**)₃), 1.18-1.27 (3H, m, CH₂C**H₂**C**H₂**C**H₂**CH₂)

HRMS (ES) 461.2660 (MH^+). $C_{25}H_{36}N_2O_6$ requires 461.2651

1-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylamino-propionylamino]cyclohexanecarboxylic acid (4.56)

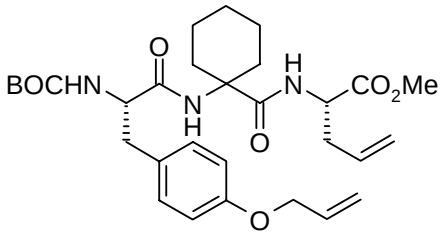


Dipeptide **4.55** (4.40g, 9.56 mmol) was dissolved in THF (50 mL) and hydrolysed using General procedure C1. This afforded a white solid, 4.18g, 98%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.15 (2H, d J=8.3Hz, Ar-**H**), 6.86 (2H, d J=8.3Hz, Ar-**H**), 6.39 (1H, bs, NH Cy), 6.04 (1H, tdd J=5.1Hz, J=9.9Hz, J=15.7Hz, OCH₂C**H**CH₂), 5.26-5.43 (2H, m, OCH₂CHC**H₂**), 5.21 (1H, d J=7.5Hz, N**H** Tyr), 4.51 (2H, d J=5.1Hz, OC**H₂**CHCH₂), 4.29-4.34 (1H, m, C**H**CH₂Ph), 3.00-3.06 (2H, m, CHC**H₂**Ph), 1.94-2.05 (2H, m, C**H₂**CH₂CH₂CH₂C**H₂**), 1.75-1.84 (2H, m, C**H₂**CH₂CH₂CH₂C**H₂**), 1.50-1.64 (3H, m, CH₂C**H₂**C**H₂**C**H₂**CH₂), 1.42 (9H s, C(C**H₃**)₃), 1.21-1.30 (3H, m, CH₂C**H₂**C**H₂**C**H₂**CH₂)

LRMS (ES) 447.3 (MH^+). $C_{24}H_{34}N_2O_6$ requires 447.3

(S)-2-({1-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]cyclohexanecarbonyl}-amino)pent-4-enoic acid methyl ester (4.57)



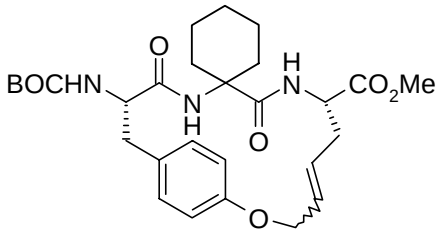
Carboxylic acid **4.56** (2.60g, 5.82 mmol) was reacted with amine **4.14** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 2.47g, 76%. R_f = 0.46 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.35 (1H, d J=7.4Hz, N**H** Gly), 7.17 (2H, d J=8.5Hz, Ar-**H**), 6.87 (2H, d J=8.5Hz, Ar-**H**), 6.04 (1H, tdd J=5.3Hz, J=10.5Hz, J=15.9Hz, OCH₂C**H**CH₂), 5.71 (1H, dddd, J=6.5Hz,

J=6.5Hz, J=10.4Hz, J=17.7Hz, $CHCH_2C\mathbf{H}CH_2$), 5.26-5.43 (2H, m, $OCH_2CHC\mathbf{H_2}$), 5.09-5.13 (3H, m, $CHCH_2CHC\mathbf{H_2}$ and N**H** Cy), 5.00 (1H, bs, N**H** Tyr), 4.60 (1H, ddd J=6.5Hz, J=6.9Hz, J=7.4Hz, $C\mathbf{H}CH_2CHCH_2$), 4.52 (2H, d J=5.3Hz, $OC\mathbf{H_2}CHCH_2$), 4.25 (1H, ddd J=7.1Hz, J=7.1Hz, J=7.0Hz, $C\mathbf{H}CH_2Ph$), 3.72 (3H, s, $CO_2C\mathbf{H_3}$), 2.99-3.08 (2H, m, $CHC\mathbf{H_2}Ph$), 2.59 (1H, ddd J=6.5Hz, J=6.5Hz, J=14.1Hz, $CHC\mathbf{H_2}CHCH_2$), 2.48 (1H, ddd J=6.9Hz, J=6.9Hz, J=14.1Hz, $CHC\mathbf{H_2}CHCH_2$), 1.94-2.08 (2H, m, $C\mathbf{H_2}CH_2CH_2CH_2C\mathbf{H_2}$), 1.71-1.85 (2H, m, $C\mathbf{H_2}CH_2CH_2CH_2C\mathbf{H_2}$), 1.46-1.59 (3H, m, $CH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}CH_2$) 1.42 (9H, s, $C(C\mathbf{H_3})_3$), 1.14-1.30 (3H, m, $CH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}CH_2$)

HRMS (ES) 558.3182 (MH^+). $C_{30}H_{43}N_3O_7$ requires 558.3179

(*E/Z*)-(7S, 13S)-10-Cyclohexyl-13-tertbutoxycarbonylamino-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (4.58)



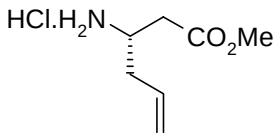
Diene **4.57** (1.00g, 1.79 mmol) was reacted with Grubbs second generation catalyst using General Procedure E2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.294g, 31%. A 1:6.5 ratio of geometric isomers was obtained R_f = 0.28 and 0.29 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm for major isomer from mixture (500 MHz in $CDCl_3$) 7.20 (2H, d J=8.0 Hz, Ar-**H**), 6.86 (2H, d J=8.0Hz, Ar-**H**), 6.52 (1H, d J=6.5Hz, N**H** Cy), 6.29 (1H, d J=8.0Hz, N**H** Gly), 5.44 (1H, d J=8.5Hz, N**H** Tyr), 5.19-5.37 (1H, m, $OCH_2C\mathbf{H}CHCH_2$), 5.09-5.20 (1H, m, and $OCH_2CHC\mathbf{H}CH_2$), 4.73 (1H, ddd, J=4.5Hz, J=4.5Hz, J=8.0Hz, $C\mathbf{H}CO_2CH_3$), 4.54-4.63 (2H, m, and $OC\mathbf{H_2}CHCHCH_2$), 4.35 (1H, ddd, J=5.7Hz, J=5.7Hz, J=8.5Hz, $C\mathbf{H}CH_2Ph$), 3.75 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.09 (1H, dd J=5.7Hz, J=12.0Hz, $CHC\mathbf{H_2}Ph$), 2.87 (1H, dd, J=12.0Hz, J=12.0Hz, $CHC\mathbf{H_2}Ph$), 2.23-2.65 (4H, m, $OCH_2CHCHC\mathbf{H_2}$ and $C\mathbf{H_2}CH_2CH_2CH_2C\mathbf{H_2}$), 1.68-1.83 (2H, m, $C\mathbf{H_2}CH_2CH_2CH_2C\mathbf{H_2}$), 1.53-1.67 (3H, m, $CH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}CH_2$), 1.39 (9H, s, $C(C\mathbf{H_3})_3$), 1.02-1.18 (3H, m, $CH_2C\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}CH_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 7.15 (2H, d J=8.0Hz, Ar-H), 6.88 (2H, d J=8.0Hz, Ar-H), 4.44 (1H, ddd J=4.3Hz, J=4.3Hz, J=9.0Hz, $C\mathbf{H}CH_2Ph$), 3.68 (3H, s, $CHCO_2C\mathbf{H_3}$)

HRMS (ES) 530.2869 (MH^+). $C_{28}H_{39}N_3O_7$ requires 530.2866

(S)-3-Amino-hex-5-enoic acid methyl ester hydrochloride (4.59)



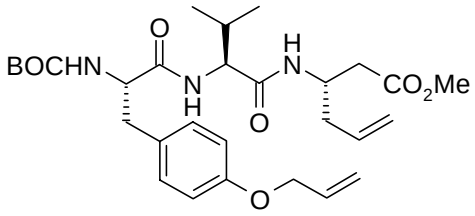
(S)-3-Amino-hex-5-enoic acid (0.500g, 3.87 mmol) was suspended in methanol (15 mL). This was esterified using General procedure F1 to afford a white solid. 0.598g, 86%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.41 (2H, bs, N**H$_2$**), 5.79 (1H, tdd, J=7.2Hz, J=7.2Hz, J=9.5, J=14.5Hz, CH_2C**H**CH_2), 5.21-5.29 (2H, m, CH_2CHC**H$_2$**), 3.73 (3H, s, CO_2C**H$_3$**), 3.64-3.65 (1H, m, C**H**$CH_2CO_2CH_3$), 2.91 (1H, dd, J=7.2Hz, J=17.2Hz, CHC**H$_2$**CO_2CH_3), 2.81 (1H, dd J=5.2, J=17.2Hz, CHC**H$_2$**CO_2CH_3) 2.69-2.74 (1H, m, CHC**H$_2$**$CHCH_2$), 2.49-2.55 (1H, m, CHC**H$_2$**$CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 170.9, 131.4, 120.7, 52.3, 48.2, 36.7, 35.6

LRMS (ES) 144.1 (MH^+). $C_7H_{14}NO_2$ requires 144.1

(S)-3-{(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-methylbutyryl-amino}-hex-5-enoic acid methyl ester (4.60)



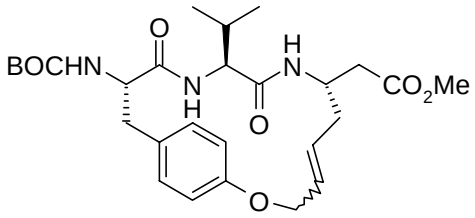
Carboxylic acid **4.13** (1.28g, 3.04 mmol) was reacted with amine **4.59** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 1.38g, 83%. R_f = 0.31 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.10 (2H, d J=8.5Hz, Ar-**H**), 6.83 (2H, d J=8.5Hz, Ar-**H**), 6.58 (1H, bs, N**H**), 6.55 (1H, d J=8.3Hz, N**H**), 6.03 (1H, tdd J=5.3Hz, J=10.5Hz, J=17.2Hz, OCH_2C**H**CH_2), 5.67-5.75 (1H, m, $CHCH_2C$**H**CH_2), 5.05-5.41 (4H, m, OCH_2CHC**H$_2$** and CH_2CHC**H$_2$**), 5.00 (1H, d J=5.8Hz, N**H**), 4.49-4.50 (2H, d J=5.3Hz, OC**H$_2$**$CHCH_2$), 4.27-4.33 (2H, m, C**H**CH_2CHCH_2 and C**H**CH_2Ph), 4.16-4.20 (1H, m, C**H**$CH(CH_3)_2$), 3.66 (3H, s, CO_2C**H$_3$**), 3.05 (1H, dd J= 6.2Hz, J=14.0Hz, CHC**H$_2$**Ph), 3.00 (1H, dd J=7.1Hz, J=14.0Hz, CHC**H$_2$**Ph), 2.53 (2H, d J=5.8Hz, CHC**H$_2$**$CHCH_2$), 2.30 (2H, dd J=6.9Hz, J=6.9Hz C**H$_2$**CO_2CH_3), 2.13-2.19 (1H, m, CHC**H**$(CH_3)_2$), 1.40 (9H, s, C(C**H$_3$**)$_3$), 0.88 (3H, d J=6.8 Hz, CHCH(C**H$_3$**)$_2$), 0.83 (3H, d J=6.8 Hz, CHCH(C**H$_3$**)$_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.3, 157.6, 133.7, 133.2, 130.2, 128.5, 188.3, 117.6, 114.9, 80.4, 68.7, 58.6, 56.0, 51.7, 45.8, 38.4, 37.7, 36.7, 30.3, 28.2, 19.1, 17.5

HRMS (ES) 546.3182 (MH^+). $C_{29}H_{43}N_3O_7$ requires 546.3179

(E/Z)-(7S,10S,13S)-13-tertButoxycarbonylamino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),4,15(19),16-tetraen-7-yl)-acetic acid methyl ester (4.61)



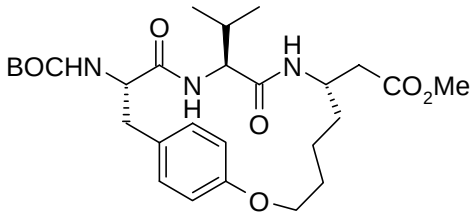
Diene **4.60** (0.700g, 1.28 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.438g, 66%. A 1:9.1 ratio of geometric isomers was obtained. R_f = 0.47 and 0.49 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR NMR for major isomer from mixture (500 MHz in $CDCl_3$) 7.04 (2H, d J=8.1Hz, Ar-**H**), 6.75 (2H, d J=8.1Hz, Ar-**H**), 6.06 (1H, d J=6.5Hz, N**H** Val), 5.95 (1H, d J=9.5Hz, N**H** Gly), 5.48-5.57 (2H, m, $OCH_2C\mathbf{H}CHCH_2$ and $OCH_2CHC\mathbf{H}CH_2$), 5.33 (1H, d J=8.3Hz, N**H** Tyr), 4.54-5.65 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.37-4.43 (1H, m, $C\mathbf{H}CH_2CO_2CH_3$), 4.14-4.22 (1H, m, $C\mathbf{H}CH_2Ph$), 3.83-3.86 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.68 (3H, s, $CO_2C\mathbf{H_3}$), 3.07 (1H, dd J=4.4Hz, J=12.1Hz, $CHC\mathbf{H_2}Ph$), 2.69 (1H, dd J=12.1Hz, J=12.1Hz, $CHC\mathbf{H_2}Ph$), 2.43-2.52 (2H, m, $C\mathbf{H_2}CO_2CH_3$), 2.27-2.29 (2H, m, $OCH_2CHCHC\mathbf{H_2}$), 1.96-2.02 (1H, m, $CHC\mathbf{H}(CH_3)_2$) 1.45 (9H, s, $C(C\mathbf{H_3})_3$), 0.82 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$), 0.79 (3H, d J=6.8 Hz, $CHCH(C\mathbf{H_3})_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 6.80 (2H, d J=8.5Hz, Ar-**H**), 6.14 (1H, d J=8.4Hz, N**H** Val), 6.00 (1H, d J=7.6Hz, N**H** Gly), 2.37-2.42 (2H, m, $C\mathbf{H_2}CO_2CH_3$)

HRMS (ES) 518.2873 (MH^+). $C_{27}H_{39}N_3O_7$ requires 518.2866

(7S,10S,13S)-13-tertButoxycarbonylamino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-7-yl)-acetic acid methyl ester (4.62)



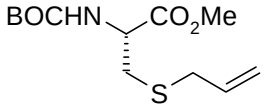
The unsaturated macrocycle **4.61** (0.440g, 0.850 mmol) was hydrogenated in 20 mL of methanol using General Procedure G1 to afford a brown solid, 0.371g, 84%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.05 (2H, d J=8.3Hz, Ar-**H**), 6.79 (2H, d J=8.3Hz, Ar-**H**), 6.31 (1H, d J=6.9Hz, N**H** Val), 5.93 (1H, d J=9.0Hz, N**H**), 5.26 (1H, d J=7.9Hz, N**H**), 4.29-4.35 (1H, m, $OC\mathbf{H_2}CH_2CH_2CH_2$), 4.21-4.26 (2H, m, $C\mathbf{H}CH_2Ph$ and $C\mathbf{H}CH_2CO_2CH_3$), 4.09 (1H, ddd J=1.1Hz, J=3.0Hz,

J=6.6Hz, OC**H**$_2$CH$_2$CH$_2$CH$_2$), 3.72 (1H, dd J=3.5Hz, J=8.6Hz, C**H**CH(CH$_3$)$_2$), 3.48 (3H, s, CO$_2$C**H**$_3$), 3.10 (1H, dd J=5.3Hz, J=12.3Hz, CHC**H**$_2$Ph) 2.65 (1H, dd J=12.3Hz, J=12.3Hz, CHC**H**$_2$Ph), 2.35-2.48 (2H, m, C**H**$_2$CO$_2$CH$_3$), 1.92-1.97 (1H, m, CHC**H**(CH$_3$)$_2$), 1.73-1.87 (2H, m, OCH$_2$C**H**$_2$CH$_2$CH$_2$), 1.44 (9H, s, C(C**H**$_3$)$_3$), 1.21-1.29 (4H, m, OCH$_2$CH$_2$C**H**$_2$CH$_2$ and OCH$_2$CH$_2$CH$_2$C**H**$_2$), 0.82 (3H, d J=6.6Hz, CHCH(C**H**$_3$)$_2$), 0.79 (3H, d J=6.6Hz, CHCH(C**H**$_3$)$_2$)

HRMS (ES) 520.3023 (MH$^+$). $C_{27}H_{41}N_3O_7$ requires 520.3023

(R)-3-Allylsulfanyl-2-tertbutoxycarbonylaminopropionic acid methyl ester (4.63) Lit[6]

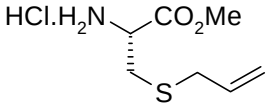


N-BOC-Cys-OMe (5.00g, 21.2 mmol) was dissolved in anhydrous DCM (30 mL) under an atmosphere of argon. To this triethylamine (3.26 mL, 1.1 equiv) and allyl bromide (2.02 mL, 1.1 equiv) were added. The mixture was stirred at rt for eighteen h before being concentrated *in vacuo*. The residue was partitioned between EtOAc and 1M HCl$_{(aq)}$. The organic phase was washed with brine, dried (MgSO$_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 3.22g, 55%. R_f = 0.19 (1/5 (EtOAc / (50/70) Pet ether).

^{1}H NMR ppm (500 MHz in CDCl$_3$) 5.65 (1H, tdd J=7.1Hz, J=10.1Hz, J=17.1Hz, CH$_2$C**H**CH$_2$), 5.34 (1H, d J=8.1Hz, N**H**), 5.03-5.10 (2H, m, CH$_2$CHC**H**$_2$), 4.41-4.43 (1H, m, C**H**CO$_2$CH$_3$), 3.76 (3H, s, CHCO$_2$C**H**$_3$), 3.05-3.09 (2H, d J=5.7Hz, C**H**$_2$CHCH$_2$), 2.86 (1H, dd J=4.5Hz, J=14.8Hz, CHC**H**$_2$S), 2.77 (1H, dd J=8.1Hz, J=14.8Hz, CHC**H**$_2$S), 1.41 (9H, s, C(C**H**$_3$)$_3$)

LRMS (ES) 276.1 (MH$^+$). $C_{12}H_{21}NO_4S$ requires 276.1

(R)-3-Allylsulfanyl-2-aminopropionic acid methyl ester hydrochloride (4.64) Lit[6]



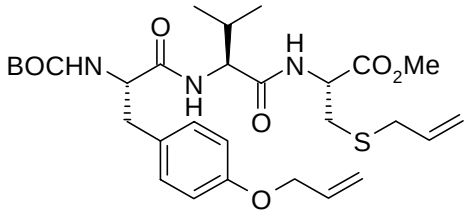
N-BOC protected compound **4.63** (3.20g, 11.6 mmol) was reacted using General Procedure I4 to afford a white solid 2.46g, 100%.

^{1}H NMR ppm (500 MHz in CD$_3$OD) 5.78 (1H, tdd J=7.2Hz, J=10.0Hz, J=17.1Hz, CH$_2$C**H**CH$_2$), 5.13-5.21 (2H, m, CH$_2$CHC**H**$_2$), 4.23 (1H, dd J=4.4Hz, J=8.1Hz, C**H**CO$_2$CH$_3$), 3.75 (3H, s, CHCO$_2$C**H**$_3$), 3.17-3.21 (2H, d J=7.2Hz, C**H**$_2$CHCH$_2$), 3.07 (1H, dd J=4.4Hz, J=14.8Hz, CHC**H**$_2$S), 2.89 (1H, dd J=8.1Hz, J=14.8Hz, CHC**H**$_2$S)

^{13}C NMR ppm (75 MHz in CD$_3$OD) 177.5, 170.2, 133.4, 52.6, 52.0, 34.1, 30.0

LRMS (ES) 176.0 (MH$^+$). $C_7H_{13}NO_2S$ requires 176.1

(R)-2-{(S)-2-[(S)-3-(4-Allyloxy-phenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-methylbutyryl-amino}-3-allylsulfanyl-propionic acid methyl ester (4.65)

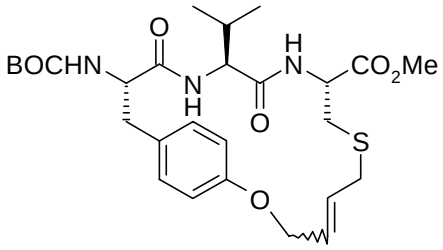


Carboxylic acid **4.13** (1.50g, 3.57 mmol) was reacted with amine **4.64** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 2.00g, 97%. R_f = 0.34 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 7.17 (2H, d J=8.4Hz, Ar-**H**), 6.82 (2H, d J=8.4Hz, Ar-**H**), 6.05 (1H, tdd J=5.3Hz, J=10.6Hz, J=17.4Hz, $OCH_2C\mathbf{H}CH_2$), 5.73-5.82 (1H, m, $SCH_2C\mathbf{H}CH_2$), 5.06-5.41 (4H, m, $OCH_2CHC\mathbf{H_2}$ and $SCH_2CHC\mathbf{H_2}$), 4.54-4.61 (1H, m, $C\mathbf{H}CO_2CH_3$), 4.49-4.53 (2H, m, $OC\mathbf{H_2}CHCH_2$), 4.22-4.36 (2H, m, $C\mathbf{H}CH_2Ph$ and $C\mathbf{H}CH(CH_3)_3$), 3.76 (3H, s, $C\mathbf{H}CO_2CH_3$), 3.15-3.21 (2H, m, $SC\mathbf{H_2}CHCH_2$), 2.74-3.04 (4H, m, $CHC\mathbf{H_2}Ph$ and $CHC\mathbf{H_2}SCH_2CHCH_2$), 2.02-2.09 (1H, m, $CHC\mathbf{H}(CH_3)_3$), 1.39 (9H, s, $C(C\mathbf{H_3})_3$), 0.96 (3H, d J=6.7Hz, $CHCH(C\mathbf{H_3})_3$), 0.94 (3H, d J=6.7Hz, $CHCH(C\mathbf{H_3})_3$)

HRMS (ES) 578.2892 (MH^+). $C_{29}H_{43}N_3O_7S$ requires 578.2900

(*E/Z*)-(9R,12S,15S)-15-tertButoxycarbonylamino-12-isopropyl-11,14-dioxo-2-oxa-7-thia-10,13-diaza-bicyclo[15.2.2]henicosa-1(20),4,17(21),18-tetraene-9-carboxylic acid methyl ester (4.66)



Diene **4.65** (1.00g, 1.73 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.685g, 72%. A 1:1.7 ratio of geometric isomers was obtained. R_f = 0.25 and 0.23 (1/1 (EtOAc / (50/70) Pet ether)).

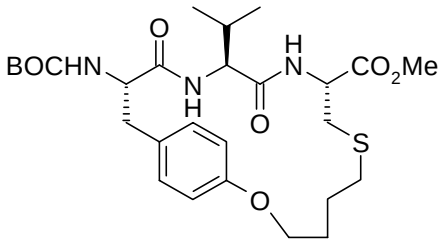
^{1}H NMR ppm for major isomer from mixture (500 MHz in $(CD_3)OD$) 7.03 (2H, d J=8.7Hz, Ar-**H**), 6.81 (2H, d J=8.7Hz, Ar-**H**), 5.67-5.81 (2H, m, $OCH_2C\mathbf{H}CHCH_2$) and $OCH_2CHC\mathbf{H}CH_2$), 4.62-4.70 (2H, m, $OC\mathbf{H_2}CH_2CH_2CH_2S$), 4.58 (1H, dd J=5.4Hz, J=14.5Hz, $C\mathbf{H}CO_2CH_3$), 4.44-4.51 (1H, m, $C\mathbf{H}CH_2Ph$), 4.18-4.29 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.69 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.08-3.22 (2H, m, $OCH_2CH_2CH_2C\mathbf{H_2}S$), 3.06-3.11 (1H, m, $CHC\mathbf{H_2}Ph$), 2.86-2.97 (1H, m, $CHC\mathbf{H_2}Ph$), 2.67-2.86 (1H, m, $CHC\mathbf{H_2}S$), 2.56-2.64 (1H, m, $CHC\mathbf{H_2}S$), 1.81-1.89

(1H, m, CHC**H**$(CH_3)_2$), 1.42 (9H, s, C($C\mathbf{H_3}$)$_3$), 0.94 (3H, d J=6.9Hz, CHCH($C\mathbf{H_3}$)$_2$), 0.92 (3H, d J=6.9Hz, CHCH($C\mathbf{H_3}$)$_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 7.11 (2H, d J=8.0Hz, Ar-**H**), 6.75 (2H, d J=8.0Hz, Ar-**H**), 0.85 (3H, d J=6.5Hz, CHCH($C\mathbf{H_3}$)$_2$), 0.80 (3H, d J=6.5Hz, CHCH($C\mathbf{H_3}$)$_2$)

HRMS (ES) 550.2599 (MH^+). $C_{27}H_{39}N_3O_7S$ requires 550.2587

(9R,12S,15S)-15-tertButoxycarbonylamino-12-isopropyl-11,14-dioxo-2-oxa-7-thia-10,13-diazabicyclo-[15.2.2]henicosa-1(20),17(21),18-triene-9-carboxylic acid methyl ester (4.67)

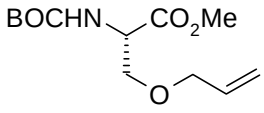


The unsaturated macrocycle **4.66** (0.700g, 1.27 mmol) was hydrogenated in 50 mL of methanol using General Procedure G2 to afford a brown solid, 0.309g, 44%

^{1}H NMR ppm (500 MHz in $(CD_3)OD$ 7.03 (2H, d J=8.2Hz, Ar-**H**), 6.76 (2H, d J=8.2Hz, Ar-**H**), 4.58 (1H, dd J=6.0Hz, J=14.4Hz, C**H**CO_2CH_3), 4.18-4.31 (3H, m, C**H**CH_2Ph and OC$\mathbf{H_2}CH_2CH_2CH_2$S), 3.98-4.02 (2H, m, $OCH_2CH_2CH_2C\mathbf{H_2}$S and C**H**$CH(CH_3)_2$), 3.84-3.89 (1H, m, $OCH_2CH_2CH_2C\mathbf{H_2}$S), 3.70 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.84-3.03 (2H, m, CHC$\mathbf{H_2}$Ph), 2.71-2.85 (2H, m, CHC$\mathbf{H_2}$S), 1.97-2.13 (1H, m, CHC**H**$(CH_3)_2$), 1.80-1.91 (2H, m, $OCH_2CH_2C\mathbf{H_2}CH_2$S), 1.73-1.79 (2H, m, $OCH_2C\mathbf{H_2}CH_2CH_2$S), 1.42 (9H, s, C($C\mathbf{H_3}$)$_3$), 0.94 (3H, d J=6.7Hz, CHCH($C\mathbf{H_3}$)$_2$), 0.82 (3H, d J=6.7Hz, CHCH($C\mathbf{H_3}$)$_2$)

HRMS (ES) 552.2739 (MH^+). $C_{27}H_{41}N_3O_7S$ requires 552.2743

(S)-3-Allyloxy-2-tertbutoxycarbonylaminopropionic acid methyl ester (4.68) Lit[7]



Allyl alcohol (1.97mL, 29.0 mmol) was dissolved in diethyl ether (20 mL). This was cooled to 0^0C and ethyl chloroformate (1.1 equiv) and triethylamine (1.1 equiv) were added. After stirring in ice for twenty min the resultant white precipitate was removed by suction filtration. The filtrate was concentrated *in vacuo* and the residue dissolved in THF (4 mL). To this a solution of allyl palladium chloride dimer (0.008 equiv) and triphenylphosphine (0.035 equiv) in THF (3 mL) was added. The mixture was stirred at rt for twenty min and this was then added to a solution of N-Boc-Ser-OMe (5.00g, 0.080 equiv) in THF (20 mL). The mixture was stirred at rt for eighteen h and then concentrated *in vacuo*. The crude material was purified by flash

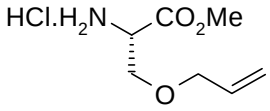
chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow oil, 1.25g, 21%. R_f = 0.27 (1/6 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 in $CDCl_3$) 5.64 (1H, tdd J=5.5Hz, J=5.5Hz, J=11.0Hz, J=16.0Hz, OCH_2CHCH_2), 5.37 (1H, d J=8.6Hz, N**H**), 4.97-5.12 (2H, m, OCH_2CHCH_2), 4.21-4.24 (1H, m, $CHCO_2CH_3$), 3.74-3.80 (2H, m, OCH_2CHCH_2), 3.65 (1H, dd J=3.2Hz, J=9.4Hz, $CHCH_2OCH_2CHCH_2$), 3.55 (3H, s, $CHCO_2CH_3$), 3.46 (1H, dd J=3.4Hz, J=9.4Hz, $CHCH_2OCH_2CHCH_2$), 1.26 (9H, s, $C(CH_3)_3$)

^{13}C NMR ppm (75 MHz in CD_3OD) 173.3, 171.4, 133.6, 118.6, 66.6, 53.1, 30.5, 26.7

LRMS (ES) 260.2 (MH^+). $C_{12}H_{21}NO_5$ requires 260.1

(S)-3-Allyloxy-2-aminopropionic acid methyl ester hydrochloride (4.69) Lit[7]

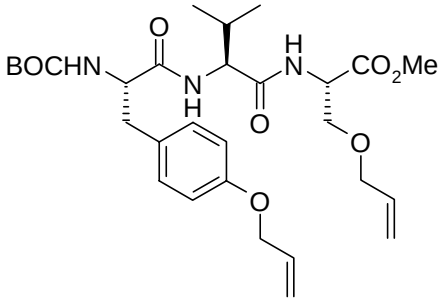


N-BOC protected compound **4.68** (1.00g, 3.86 mmol) was reacted using General Procedure I1 to afford a white solid 0.656g, 87%.

^{1}H NMR ppm (500 MHz in CD_3OD) 5.84-5.92 (1H, m, OCH_2CHCH_2), 5.19-5.31 (2H, m, OCH_2CHCH_2), 4.27 (1H, dd J=3.2Hz, J=4.6Hz, $CHCO_2CH_3$), 3.99-4.09 (2H, m, OCH_2CHCH_2), 3.85-3.91 (1H, m, $CHCH_2OCH_2$), 3.83 (3H, s, $CHCO_2CH_3$), 3.79 (1H, dd J=3.2Hz, J=10.6Hz, $CHCH_2OCH_2$)

LRMS (ES) 160.1 (MH^+). $C_7H_{13}NO_3$ requires 160.1

(S)-3-Allyloxy-2-{(S)-2-[(S)-3-(4-allyloxy-phenyl)-2-tertbutoxycarbonylamino-propionylamino]-3-methyl-butyrylamino}propionic acid methyl ester (4.70)



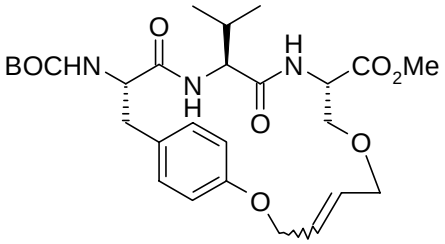
Carboxylic acid **4.13** (1.29g, 3.07 mmol) was reacted with amine **4.69** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 1.07g, 62%. R_f = 0.46 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 7.12 (2H, d J=8.0 Hz, Ar-**H**), 6.82 (2H, d J=8.0Hz, Ar-**H**), 6.04 (1H, tdd J=5.3Hz, J=5.3Hz, J=10.4Hz, J=17.1Hz, $PhOCH_2CHCH_2$), 5.87 (1H, dddd J=5.5Hz, J=5.6Hz, J=11.0Hz, J=16.0Hz, $CH_2OCH_2CHCH_2$), 5.13-5.40 (4H, m, $PhOCH_2CHCH_2$ and $CH_2OCH_2CHCH_2$,), 4.59 (1H, dd, J=3.2Hz, J=4.7Hz, $CHCO_2CH_3$), 4.46-4.53 (2H, m, $PhOCH_2CHCH_2$), 4.25-4.32 (2H, m, $CHCH_2Ph$ and

C**H**CH$(CH_3)_2$), 4.02 (1H, dd J=5.5Hz, J=13.0Hz, $CH_2OC\mathbf{H_2}CHCH_2$), 3.97 (1H, dd J=5.6Hz, J=13.0Hz, $CH_2OC\mathbf{H_2}CHCH_2$), 3.83 (1H, dd J=4.7Hz, J=9.8Hz, $C\mathbf{H_2}OCH_2CHCH_2$), 3.72 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.67 (1H, dd J=3.8Hz, J=8.7Hz, $C\mathbf{H_2}OCH_2CHCH_2$), 3.02 (1H, dd J=5.5Hz, J=13.9Hz, CHC$\mathbf{H_2}$Ph), 2.75 (1H, dd J=8.9Hz, J=13.9Hz, CHC$\mathbf{H_2}$Ph), 1.95-2.06 (1H, m, CHC**H**$(CH_3)_2$), 1.37 (9H, s, $C(C\mathbf{H_3})_3$), 0.98 (3H, d J=6.7 Hz, $CHCH(C\mathbf{H_3})_2$), 0.94 (3H, d J=6.7 Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 562.3121 (MH^+). $C_{29}H_{43}N_3O_8$ requires 562.3128

(E/Z)-(9S,12S,15S)-15-tertButoxycarbonylamino-12-isopropyl-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo-[15.2.2]henicosa-1(20),4,17(21),18-tetraene-9-carboxylic acid methyl ester (4.71)



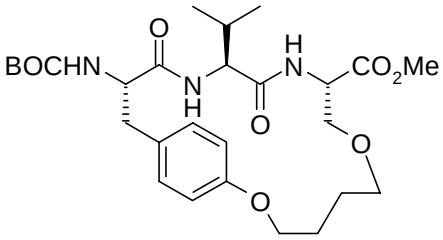
Diene **4.70** (1.06g, 1.89 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.233g, 23%. A 1:4.7 ratio of geometric isomers was obtained. R_f = 0.28 and 0.27 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm for major isomer from mixture (500 MHz in $CDCl_3$) 7.09 (2H, d J=8.4Hz, Ar-**H**), 6.77 (2H, d J=8.4Hz, Ar-**H**), 6.27 (1H, d J=8.3Hz, N**H** Val), 6.24 (1H, d J=8.3Hz, N**H** Ser), 5.73 (1H, ddd J=6.5Hz, J=6.7Hz, J=7.5Hz, $PhOCH_2CHC\mathbf{H}CH_2$), 5.62 (1H, td J=6.1Hz, J=6.1Hz, J=7.5Hz, $PhOCH_2C\mathbf{H}CHCH_2$), 5.40 (1H, d J=8.6Hz, N**H** Tyr), 4.76 (1H, ddd J=4.0Hz, J=4.3Hz, J=8.3Hz, C**H**CO_2CH_3), 4.67-4.61 (2H, m, $PhOC\mathbf{H_2}CHCHCH_2$), 4.36 (1H, ddd J=4.2Hz, J=8.6Hz, J=9.6Hz, C**H**CH_2Ph), 4.13 (1H, dd J=6.7Hz, J=13.6Hz, $CH_2OC\mathbf{H_2}CHCHCH_2$), 4.09 (1H, dd J=6.5Hz, J=13.6Hz, $CH_2OC\mathbf{H_2}CHCHCH_2$), 3.80 (1H, dd J=7.7Hz, J=8.3Hz, C**H**$CH(CH_3)_2$), 3.78 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.58 (1H, dd J=4.3Hz, J=9.1Hz, $C\mathbf{H_2}OCH_2CHCHCH_2$), 3.51 (1H, dd J=4.0Hz, J=9.1Hz, $C\mathbf{H_2}OCH_2CHCHCH_2$), 3.07 (1H, dd J=9.6Hz, J=13.7Hz, CHC$\mathbf{H_2}$Ph), 2.91 (1H, dd J=4.2Hz, J=13.7Hz, CHC$\mathbf{H_2}$Ph), 1.99-2.08 (1H, m, CHC**H**$(CH_3)_2$), 1.46 (9H, s, $C(C\mathbf{H_3})_3$), 0.88 (3H, d J=6.7Hz, $CHCH(C\mathbf{H_3})_2$), 0.88 (3H, d J=6.7 Hz, $CHCH(C\mathbf{H_3})_2$)

Selected ^{1}H-NMR for minor isomer from mixture: 6.80 (2H, d J=8.5 Hz, Ar-**H**), 4.33-4.25 (1H, m, C**H**CH_2Ph), 3.01 (1H, dd J=4.3Hz, J=12.9Hz, CHC$\mathbf{H_2}$Ph), 0.85 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$), 0.82 (3H, d J=6.5Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 534.2820 (MH^+). $C_{27}H_{39}N_3O_8$ requires 534.2815

(9S,12S,15S)-15-tertButoxycarbonylamino-12-isopropyl-11,14-dioxo-2,7-dioxa-10,13-diazabicyclo-[15.2.2]henicosa-1(20),17(21),18-triene-9-carboxylic acid methyl ester (4.72)

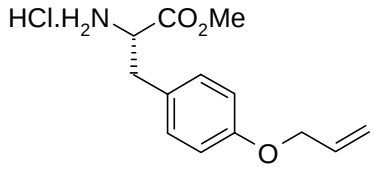


The unsaturated macrocycle **4.71** (0.200g, 0.375 mmol) was hydrogenated in 20 mL of methanol using General Procedure G2 to afford a brown solid, 0.200g, 100%

^{1}H NMR ppm (500 MHz in CD_3OD) 7.05 (2H, d J=8.3Hz, Ar-**H**), 6.75 (2H, d J=8.3Hz, Ar-**H**), 4.60 (1H, dd J=3.9Hz, J=6.3Hz, C**H**CO_2CH_3), 4.21-4.36 (2H, m, C**H**CH_2Ph and PhOC**H$_2$**$CH_2CH_2CH_2$), 4.02-4.17 (3H, m, C**H**$CH(CH_3)_2$ and PhOC**H$_2$**$CH_2CH_2CH_2$ and CH_2OC**H$_2$**$CH_2CH_2CH_2$), 3.86-3.93 (1H, m, CH_2OC**H$_2$**$CH_2CH_2CH_2$), 3.69 (3H, s, $CHCO_2$C**H$_3$**), 3.47-3.55 (2H, m, C**H$_2$**$OCH_2CH_2CH_2CH_2$), 2.80-2.91 (2H, m, CHC**H$_2$**Ph), 1.92-2.05 (1H, m, CHC**H**$(CH_3)_2$), 1.71-1.90 (1H, m, PhOCH_2C**H$_2$**CH_2CH_2), 1.55-1.71 (3H, m, PhOCH_2C**H$_2$**CH_2CH_2 and PhOCH_2CH_2C**H$_2$**CH_2), 1.44 (9H, s, C(C**H$_3$**)$_3$), 0.87 (3H, d J=6.4 Hz, CHCH(C**H$_3$**)$_2$), 0.86 (3H, d J=6.4Hz, CHCH(C**H$_3$**)$_2$)

HRMS (ES) 536.2979 (MH^+). $C_{27}H_{41}N_3O_8$ requires 536.2972

(S)-3-(4-Allyloxyphenyl)-2-amino-propionic acid methyl ester hydrochloride (4.73)



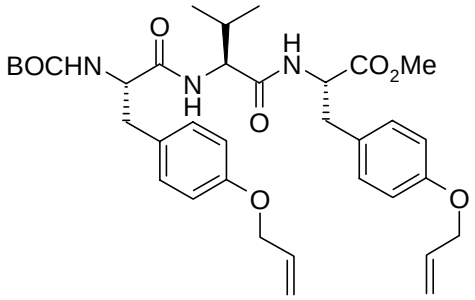
N-BOC-O-allyl-Tyr-H (5.00g, 16.3 mmol) was suspended in methanol (30 mL). This was simultaneously esterified and N-deprotected using General procedure J to afford a white solid. 4.23g, 96%.

^{1}H NMR ppm (500 MHz in CD_3OD) 7.13 (2H, d J=8.6Hz, Ar-**H**), 6.91 (2H, d J=8.6Hz, Ar-**H**), 6.03 (1H, tdd, J= 5.1Hz, J=5.1Hz, J=10.4Hz, J=17.1Hz, OCH_2C**H**CH_2), 5.21-5.39 (2H, m, OCH_2CHC**H$_2$**) 4.51-4.53 (2H, m, OC**H$_2$**$CHCH_2$), 4.22-4.25 (1H, m, C**H**CH_2Ph), 3.79 (3H, s, CO_2C**H$_3$**), 3.17 (1H, dd J=5.9Hz, J=14.5Hz, CHC**H$_2$**Ph), 3.07 (1H, dd J=7.5Hz, J=14.5Hz, CHC**H$_2$**Ph)

^{13}C NMR ppm (75 MHz in CD_3OD) 170.5, 159.8, 134.9, 131.6, 127.2, 117.5, 116.4, 69.8, 55.3, 53.6, 36.6

HRMS (ES) 236.1428 (MH^+). $C_{13}H_{17}NO_3$ requires 236.1286

(S)-3-(4-Allyloxyphenyl)-2-{(S)-2-[(S)-3-(4-allyloxyphenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-methyl-butyrylamino}propionic acid methyl ester (4.74)

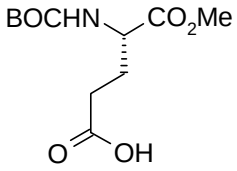


Carboxylic acid **4.13** (1.50g, 3.57 mmol) was reacted with amine **4.73** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.370g, 17%. R_f = 0.78 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.10 (2H, d J=7.8Hz, Ar-**H**), 7.00 (2H, d J=7.8Hz, Ar-**H**), 6.84 (2H, d J=7.3 Hz, Ar-**H**), 6.83 (2H, d J=7.3 Hz, Ar-**H**), 6.46 (1H, d J=8.1Hz, N**H**), 6.20 (1H, d J=6.9Hz, N**H**), 5.99-6.08 (2H, m, $OCH_2C\mathbf{H}CH_2$), 5.26-5.41 (4H, m, $OCH_2CHC\mathbf{H_2}$), 4.92-4.97 (1H, m, $C\mathbf{H}CO_2CH_3$), 4.74-4.79 (1H, m, $C\mathbf{H}CH_2Ph$), 4.47-4.50 (1H, m, $OC\mathbf{H_2}CHCH_2$), 4.24-4.31 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.71 (3H, s, $CO_2C\mathbf{H_3}$), 2.96-3.06 (4H, m, $CHC\mathbf{H_2}Ph$), 2.06-2.10 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.41 (9H, s, $C(C\mathbf{H_3})_3$), 0.86 (3H, d J=6.5Hz, $CHCH(C\mathbf{H_3})_2$), 0.80 (3H, d J=6.5Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 638.3447 (MH^+). $C_{35}H_{47}N_3O_8$ requires 638.3441

(S)-2-tertButoxycarbonylaminopentanedioic acid-1-methyl ester (4.76) Lit[8]

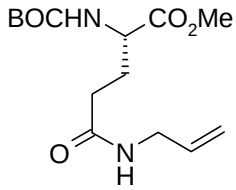


Glu-OMe (2.50g, 15.5 mmol) was N-BOC protected using General Procedure K to afford a white solid, 4.05g, 97%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 10.41 (1H, bs, $CO_2\mathbf{H}$), 5.23 (1H, d J=8.0Hz, N**H**), 4.32-4.37 (1H, m, $C\mathbf{H}CO_2CH_3$), 3.75 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.40-2.52 (2H, m, $CHCH_2C\mathbf{H_2}$), 2.16-2.23 (1H, m, $CHC\mathbf{H_2}CH_2$), 1.92-2.00 (1H, m, $CHC\mathbf{H_2}CH_2$), 1.44 (9H, s, $C(C\mathbf{H_3})_3$)

LRMS (ES) 262.1 (MH^+). $C_{11}H_{19}NO_6$ requires 262.1

(S)-4-Allylcarbamoyl-2-tertbutoxycarbonylaminobutyric acid methyl ester (4.77)

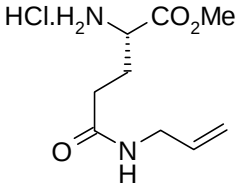


Carboxylic acid **4.76** (4.05g, 15.5 mmol) was reacted with allyl amine using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 0.652g, 14%. R_f = 0.39 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 8.06 (1H, bs, N**H**CH_2CHCH_2), 6.98 (1H, d J=7.6Hz, N**H**$CHCO_2CH_3$), 5.78-5.87 (1H, m, NCH_2C**H**CH_2), 5.06-5.20 (2H, m, NCH_2CHC**H$_2$**), 4.13 (1H, ddd J=4.8Hz, J=7.6Hz, J=12.4Hz, C**H**CO_2CH_3), 3.78 (2H, d J=4.3Hz, NC**H$_2$**$CHCH_2$), 3.71 (3H, s, CO_2C**H$_3$**), 2.30 (2H, dd J=7.4Hz, J=7.4Hz $CHCH_2C$**H$_2$**), 2.08-2.14 (1H, m, CHC**H$_2$**CH_2), 1.85-1.93 (1H, m, CHC**H$_2$**CH_2), 1.43 (9H, s, C(C**H$_3$**)$_3$)

LRMS (ES) 301.2 (MH^+). $C_{14}H_{24}N_2O_5$ requires 301.2

(S)-4-Allylcarbamoyl-2-aminobutyric acid methyl ester hydrochloride (4.78)

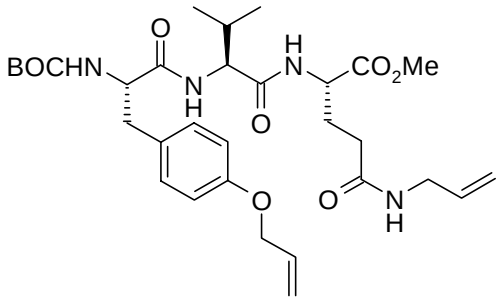


N-BOC protected compound **4.77** (0.800g, 2.66 mmol) was reacted using General Procedure I4 to afford a white solid, 0.533g, 100%

^{1}H NMR ppm (500 MHz in CD_3OD) 5.75-5.83 (1H, m, NCH_2C**H**CH_2), 5.04-5.17 (2H, m, NCH_2CHC**H$_2$**), 4.03-4.09 (1H, m, C**H**CO_2CH_3), 3.79 (3H, s, CO_2C**H$_3$**), 3.75 (2H, d J=3.8Hz, C**H$_2$**$CHCH_2$), 2.44 (2H, dd J=7.8Hz, J=15.0Hz, $CHCH_2C$**H$_2$**), 2.07-2.20 (2H, m, CHC**H$_2$**CH_2)

LRMS (ES) 201.1 (MH^+). $C_9H_{16}N_2O_3$ requires 201.1

(S)-4-Allylcarbamoyl-2-{(S)-2-[(S)-3-(4-allyloxy-phenyl)-2-tertbutoxycarbonylaminopropionylamino]-3-methylbutyrylamino}butyric acid methyl ester (4.79)

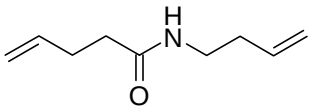


Carboxylic acid **4.13** (1.01g, 2.41 mmol) was reacted with amine **4.78** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.550g, 38%. R_f = 0.39 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.08-7.16 (3H, m, Ar-**H** and N**H** Gln), 6.84 (2H, d J=8.1Hz, Ar-**H**), 6.64 (1H, d J=6.9Hz, N**H** Val), 6.46 (1H, bs, N**H**CH_2CHCH_2), 6.00-6.08 (1H, m, OCH_2C**H**CH_2), 5.78-5.86 (1H, m, $NHCH_2C$**H**CH_2), 5.10-5.42 (4H, m, $NHCH_2CHC$**H$_2$** and OCH_2CHC**H$_2$**), 4.98 (1H, d J=6.9Hz, N**H** Tyr), 4.50-4.52 (3H, m, OC**H$_2$**$CHCH_2$ and C**H**CO_2CH_3), 4.29-4.35 (1H, m, C**H**CH_2Ph), 4.19 (1H, dd J=6.9Hz, J=7.6Hz, C**H**$CH(CH_3)_2$), 3.84-3.87 (2H, m, NHC**H$_2$**$CHCH_2$), 3.73 (3H, s, $CHCO_2C$**H$_3$**), 3.03 (2H, d J=6.0Hz, CHC**H$_2$**Ph), 2.21-2.31 (2H, m, $CHCH_2C$**H$_2$**C(O)NH), 2.06-2.13 (1H, m, CHC**H**$(CH_3)_2$), 1.99 (2H, m, CHC**H$_2$**CH_2C(O)NH), 1.40 (9H, s, $C(C$**H$_3$**$)_3$), 0.92 (3H, d J=6.7Hz, $CHCH(C$**H$_3$**$)_2$), 0.88 (3H, d J=6.7Hz, $CHCH(C$**H$_3$**$)_2$)

HRMS (ES) 603.3391 (MH^+). $C_{31}H_{46}N_4O_8$ requires 603.3394

Pent-4-enoic acid but-3-enylamide (4.91)



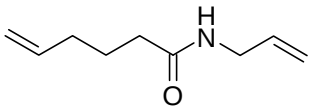
4-pentenoic acid (0.510 mL, 4.99 mmol) was reacted with **4.98** using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 0.597g, 78%. R_f = 0.28 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 5.73-5.86 (2H, m, $NHCH_2CH_2C$**H**CH_2 and CH_2CH_2C**H**CH_2) 4.95-5.09 (4H, m, $NHCH_2CH_2CHC$**H$_2$** and CH_2CH_2CHC**H$_2$**), 3.22 (2H, dt J=3.8Hz, J=6.9Hz, NHC**H$_2$**CH_2CHCH_2), 2.32-2.34 (2H, m, C**H$_2$**CH_2CHCH_2), 2.22-2.27 (4H, m, CH_2C**H$_2$**$CHCH_2$ and $NHCH_2C$**H$_2$**$CHCH_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 173.9, 136.9, 135.4, 115.7, 114.5, 38.5, 35.1, 33.5, 29.7

LRMS (ES) 154.1 (MH^+). $C_9H_{15}NO$ requires 154.1

Hex-5-enoic acid allylamide (4.93)



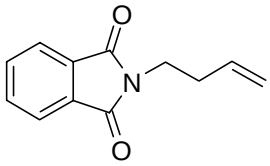
5-hexenoic acid (0.520 mL, 4.38 mmol) was reacted with allyl amine using General Procedure A1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 0.503g, 75%. R_f = 0.29 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 8.05 (1H, bs, N**H**), 5.73-5.84 (2H, m, $NHCH_2C\mathbf{H}CH_2$ and $CH_2CH_2CH_2C\mathbf{H}CH_2$), 4.90-5.16 (4H, m, $NHCH_2CHC\mathbf{H_2}$ and $CH_2CH_2CH_2CHC\mathbf{H_2}$), 3.76 (2H, ddd J=1.2Hz, J=5.7Hz, J=5.7Hz, $NHC\mathbf{H_2}CHCH_2$), 2.15-2.20 (2H, m, $C\mathbf{H_2}CH_2CH_2CHCH_2$), 2.07 (2H, dt J=1.1Hz, J=7.7Hz, J=7.8Hz, $CH_2CH_2C\mathbf{H_2}CHCH_2$), 1.64-1.70 (2H, m, $CH_2C\mathbf{H_2}CH_2CHCH_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 174.4, 137.8, 134.3, 14.8, 114.3, 41.4, 35.1, 33.0, 25.0

LRMS (ES) 154.1 (MH^+). $C_9H_{15}NO$ requires 154.1

(R)-2-But-3-enyl-isoindole-1,3-dione (4.97) Lit[9]



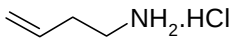
Phthalimide (10.0g, 68.0 mmol) was suspended in ethanol (100 mL) and potassium hydroxide (1 equiv) was added. This was stirred at rt for two h before being concentrated *in vacuo*. The residue was suspended in anhydrous DMF under an atmosphere of argon and 4-bromo-1-butene (0.66 equiv) was added. The mixture was heated at reflux for seventy two h, cooled, diluted with EtOAc (300 mL) and partitioned with saturated $NaHCO_{3(aq)}$. The organic phase was washed again with saturated $NaHCO_{3(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 13.1g, 96%. R_f = 0.21 (1/7 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHZ in $CDCl_3$) 7.76-7.78 (2H, m, Ar-**H**), 7.64-7.67 (2H, m, Ar-**H**), 5.80 (1H, tdd J=6.9Hz, J=10.2Hz, J=17.1Hz, $CH_2CH_2C\mathbf{H}CH_2$), 4.94-5.04 (2H, m, $CH_2CH_2CHC\mathbf{H_2}$), 3.72 (2H, t J=7.1Hz, $C\mathbf{H_2}CH_2CHCH_2$), 2.45 (2H, ddd J=6.9Hz, J=7.0Hz, J=7.0Hz, $CH_2C\mathbf{H_2}CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 168.2, 134.4, 133.8, 132.0, 123.1, 117.4, 37.2, 32.7

LRMS (ES) 202.1 (MH^+). $C_{12}H_{11}NO_2$ requires 202.1

But-3-enylamine hydrochloride (4.98) Lit[9]



Method 1

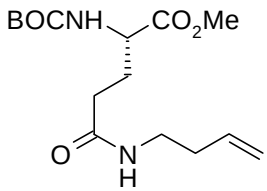
Isoindole **4.97** (5.00g, 24.9 mmol) was dissolved in ethanol (100 mL) and heated to 50°C. Hydrazine monohydrate (2 equiv) was added and the mixture was stirred at 50°C for a further one h. 1M $HCl_{(aq)}$ (30 mL) was added and this was then concentrated *in vacuo*. The residue was partitioned between diethyl ether and 1M $NaOH_{(aq)}$. The organic phase was washed with brine, dried ($MgSO_4$) and filtered. To the filtrate 2M hydrogen chloride in diethyl ether (20 mL) was added and this was concentrated *in vacuo* to afford a white solid, 0.981g, 37%.

Method 2

1M lithium aluminium hydride in diethyl ether (74.5 mL, 74.5 mmol) was dissolved in diethyl ether (75 mL) under an atmosphere of argon. The mixture was cooled in ice, aluminium trichloride (1 equiv) added portionwise and stirred in ice for ten min before allyl cyanide (1 equiv) was added. The mixture was stirred in ice for one h and then at rt for a further eighteen h. The reaction was quenched with 1M $NaOH_{(aq)}$ (10 mL) and then concentrated *in vacuo*. The residue was partitioned between diethyl ether and 1M $NaOH_{(aq)}$. The aqueous phase was extracted three more times with diethyl ether. The combined organic extracts were washed with brine, dried ($MgSO_4$) and filtered. To the filtrate 4M hydrogen chloride in 1,4-dioxane (10 mL) was added and this concentrated *in vacuo* to afford a white solid, 4.29g, 54%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.47 (2H, bs, N**H$_2$**), 5.74-5.84 (1H, m, CH_2CH_2C**H**CH_2), 5.17-5.27 (2H, m, CH_2CH_2CHC**H$_2$**), 3.10 (2H, ddd J=6.3Hz, J=8.4Hz, J=12.7Hz, C**H$_2$**CH_2CHCH_2), 2.46-2.49 (2H, m, CH_2C**H$_2$**$CHCH_2$)

(S)-4-But-3-enylcarbamoyl-2-tertbutoxycarbonylaminobutyric acid methyl ester (4.99)



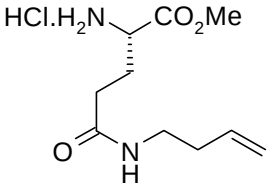
Carboxylic acid **4.76** (5.70g, 21.8 mmol) was reacted with amine **4.98** using General Procedure B1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 1.23g, 18%. R_f = 0.12 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 6.66 (1H, bs, N**H**$CH_2CH_2CHCH_2$), 5.76 (1H, tdd J=6.8Hz, J=10.2Hz, J=17.0Hz, CH_2CH_2C**H**CH_2), 5.52 (1H, d J=6.8Hz, N**H**$CHCO_2CH_3$), 5.05-5.11 (2H, m, CH_2CH_2CHC**H$_2$**), 4.11-4.17 (1H, m, C**H**CO_2CH_3), 3.68 (3H, s, $CHCO_2C$**H$_3$**), 3.27-3.49 (1H, m, NHC**H$_2$**CH_2CHCH_2), 3.19-3.25 (1H, m, NHC**H$_2$**CH_2CHCH_2), 2.37-2.51 (2H, m, $CHCH_2C$**H$_2$**C(O)), 2.18-2.23 (2H, m, $NHCH_2C$**H$_2$**$CHCH_2$), 2.10-2.15 (1H, m, CHC**H$_2$**CH_2C(O)), 1.93 (1H, dt J=7.6Hz, J=14.6Hz, CHC**H$_2$**CH_2C(O)), 1.43 (9H, s, C(C**H$_3$**)$_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 173.6, 171.4, 155.7, 135.0, 79.8, 53.6, 51.7, 38.5, 33.6, 30.2, 28.2, 28.0

LRMS (ES) 315.2 (MH^+). $C_{15}H_{26}N_2O_5$ requires 315.2

(S)-2-Amino-4-but-3-enylcarbamoylbutyric acid methyl ester hydrochloride (4.100)

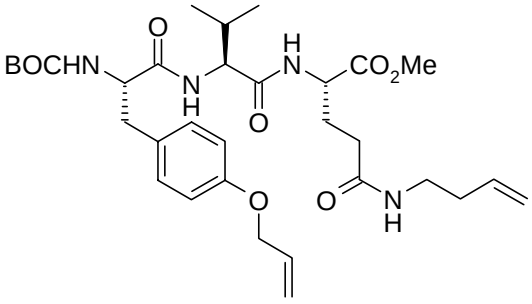


N-BOC protected compound **4.99** (1.00g, 3.18 mmol) was reacted using General Procedure I5 to afford a white solid, 0.795g, 100%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.30 (3H, bs, N**H$_2$** and N**H**$CH_2CH_2CHCH_2$), 5.78 (1H, tdd J=6.6Hz, J=10.2Hz, J=13.3Hz, CH_2CH_2C**H**CH_2), 5.01-5.12 (2H, m, CH_2CH_2CHC**H$_2$**), 4.39-4.45 (1H, m, C**H**CO_2CH_3), 3.67 (3H, s, $CHCO_2C$**H$_3$**), 3.40-3.47 (1H, m, NHC**H$_2$**CH_2CHCH_2), 3.19-3.24 (1H, m, NHC**H$_2$**CH_2CHCH_2), 2.56-2.68 (2H, m, $CHCH_2C$**H$_2$**C(O)), 2.22-2.38 (4H, m, $NHCH_2C$**H$_2$**$CHCH_2$ and CHC**H$_2$**CH_2C(O))

LRMS (ES) 215.1 (MH^+). $C_{10}H_{18}N_2O_3$ requires 215.1

(S)-2-{(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-tertbutoxycarbonylamino-propionylamino]-3-methyl-butyrylamino}-4-but-3-enylcarbamoylbutyric acid methyl ester (4.101)



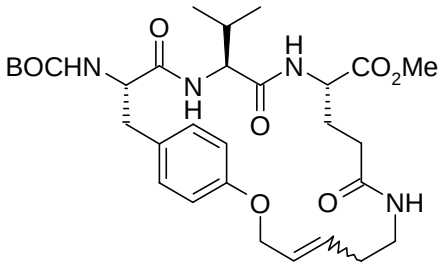
Carboxylic acid **4.13** (1.43g, 3.40 mmol) was reacted with amine **4.100** using General Procedure B2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 1.13g, 54%. R_f = 0.17 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 8.03 (1H, d J=8.0Hz, N**H** Val), 7.89 (1H, dd J=5.6Hz, J=5.6Hz, N**H**$CH_2CH_2CHCH_2$), 7.75 (1H, d J=8.5Hz, N**H**$CHCO_2CH_3$), 7.15 (2H, d J=8.4Hz, Ar-**H**), 6.96 (1H, d J=8.4Hz, N**H** Tyr), 6.83 (2H, d J=8.4Hz, Ar-**H**), 6.02 (1H, tdd J=4.9Hz, J=10.4, J=17.Hz, OCH_2C**H**CH_2), 5.74 (1H, tdd

J=6.6Hz, J=6.6Hz, J=10.1Hz, J=17.0Hz, $NHCH_2CH_2C\mathbf{H}CH_2$), 5.21-5.41 (2H, m, $OCH_2CHC\mathbf{H_2}$), 4.96-5.07 (2H, m, $NHCH_2CH_2CHC\mathbf{H_2}$), 4.51 (2H, d J=4.9Hz, $OC\mathbf{H_2}CHCH_2$), 4.22 (2H, m, $C\mathbf{H}CO_2CH_3$ and $C\mathbf{H}CH(CH_3)_2$), 4.12 (1H, ddd J=5.9Hz, J=8.4Hz, J=11.3Hz, $C\mathbf{H}CH_2Ph$), 3.56 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.04-3.18 (2H, m, $NHC\mathbf{H_2}CH_2CHCH_2$), 2.87 (1H, dd, J=11.3Hz, J=14.8Hz, $CHC\mathbf{H_2}Ph$), 2.67 (1H, dd J=5.9Hz, J=14.8Hz, $CHC\mathbf{H_2}Ph$), 2.29 (2H, dd J=7.8Hz, J=7.8Hz, $CHCH_2C\mathbf{H_2}C(O)$), 2.11-2.17 (2H, m, $NHCH_2C\mathbf{H_2}CHCH_2$), 1.85-2.00 (2H, m, $CHC\mathbf{H}(CH_3)_2$ and $CHC\mathbf{H_2}CH_2C(O)$), 1.71-1.82 (1H, m, , $CHC\mathbf{H_2}CH_2C(O)$), 1.30 (9H, s, $C(C\mathbf{H_3})_3$), 0.85 (3H, d J=7.2Hz, $CHCH(C\mathbf{H_3})_2$), 0.83 (3H, d J=7.2Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 617.3556 (MH^+). $C_{32}H_{48}N_4O_8$ requires 617.3550

(*E/Z*)-(12S,15S,18S)-18-tert-Butoxycarbonylamino-15-isopropyl-9,14,17-trioxo-2-oxa-8,13,16-triaza-bicyclo[18.2.2]tetracosa-1(23),4,20(24),21-tetraene-12-carboxylic acid methyl ester (4.102)



Diene **5.92** (1.10g, 1.78 mmol) was reacted with Grubbs second generation catalyst using General Procedure E1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.178g, 17%. %. A 1:2 ratio of geometric isomers was obtained. R_f = 0.12 (2/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR NMR for major isomer from mixture (500 MHz in CD_3OD) 7.09 (2H, d J=8.5Hz, Ar-**H**), 6.78 (2H, d J=8.5Hz, Ar-**H**), 5.69-5.71 (2H, m, $OCH_2C\mathbf{H}CHCH_2CH_2$ and $OCH_2CHC\mathbf{H}CH_2CH_2$), 4.46-4.65 (2H, m, $OC\mathbf{H_2}CHCHCH_2CH_2$ and $C\mathbf{H}CH_2Ph$), 4.20-4.36 (2H, m, $C\mathbf{H}CO_2CH_3$), 3.90-3.94 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.69 (3H, s, $CHCO_2C\mathbf{H_3}$), 2.97-3.04 (2H, m, $OCH_2CHCHCH_2C\mathbf{H_2}$), 2.91-2.95 (1H, m, $CHC\mathbf{H_2}Ph$), 2.77-2.81 (1H, m, $CHC\mathbf{H_2}Ph$), 2.27-2.33 (2H, m, $OCH_2CHCHC\mathbf{H_2}CH_2$), 2.12-2.17 (2H, m, $CHCH_2C\mathbf{H_2}C(O)$), 1.99-2.08 (2H, m, $CHC\mathbf{H}(CH_3)_2$ and $CHC\mathbf{H_2}CH_2C(O)$), 1.86-1.94 (1H, m, $CHC\mathbf{H_2}CH_2C(O)$), 1.43 (9H, s, $C(C\mathbf{H_3})_3$), 0.92 (3H, d J=6.8Hz, $CHCH(C\mathbf{H_3})_2$), 0.88 (3H, d J = 6.8Hz, $CHCH(C\mathbf{H_3})_2$)

Selected ^{1}H-NMR NMR for minor isomer from mixture: 6.99 (2H, d J=8.5 Hz, Ar-**H)**, 3.65 (3H, s, $CHCO_2C\mathbf{H_3}$), 0.84 (3H, d J=6.4Hz, $CHCH(C\mathbf{H_3})_2$), 0.81 (3H, d J=6.4Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 589.3241 (MH^+). $C_{30}H_{44}N_4O_8$ requires 589.3237

References for Chapter 4 Experimental

1. Kaul, R.; Surprenant, S.; Lubell, W. D., Systematic Study of the Synthesis of Macrocyclic Dipeptide beta-Turn Mimics Possessing 8-, 9-, and 10- Membered Rings by Ring-Closing Metathesis. *Journal of Organic Chemistry* **2005,** 70, (10), 3838-3844.

2. Griesbeck, A. G.; Hirt, J., A photochemical route to vinylglycine and a vinylglycine dipeptide. *Liebigs Annalen* **1995**, (11), 1957-61.

3. Meffre, P.; Vo-Quang, L.; Vo-Quang, Y.; Le Goffic, F., N-(Benzyloxycarbonyl)-L-vinylglycine methyl ester from L-methionine methyl ester hydrochloride. *Synthetic Communications* **1989,** 19, (20), 3457-68.

4. Nudelman, A.; Marcovici-Mizrahi, D.; Nudelman, A.; Flint, D.; Wittenbach, V., Inhibitors of biotin biosynthesis as potential herbicides. *Tetrahedron* **2004,** 60, (8), 1731-1748.

5. Belokon, Y. N.; Tararov, V. I.; Maleev, V. I.; Savel'eva, T. F.; Ryzhov, M. G., Improved procedures for the synthesis of (S)-2-[N-(N'-benzyl-prolyl)amino]benzophenone (BPB) and Ni(II) complexes of Schiff's bases derived from BPB and amino acids. *Tetrahedron: Asymmetry* **1998,** 9, (23), 4249-4252.

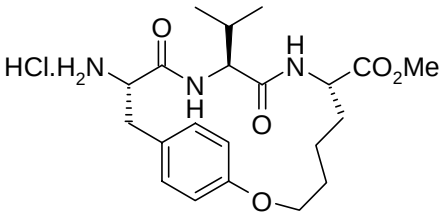
6. Goudreau, N.; Brochu, C.; Cameron, D. R.; Duceppe, J.-S.; Faucher, A.-M.; Ferland, J.-M.; Grand-Maitre, C.; Poirier, M.; Simoneau, B.; Tsantrizos, Y. S., Potent Inhibitors of the Hepatitis C Virus NS3 Protease: Design and Synthesis of Macrocyclic Substrate-Based beta-Strand Mimics. *Journal of Organic Chemistry* **2004,** 69, (19), 6185-6201.

7. Kazmaier, U.; Maier, S.; Zumpe, F. L., A journey from amino acid and peptide chemistry to palladium and back. *Synlett* **2000**, (11), 1523-1535.

8. Manesis, N. J.; Goodman, M., Synthesis of a novel class of peptides: dilactam-bridged tetrapeptides. *Journal of Organic Chemistry* **1987,** 52, (24), 5331-41.

9. McNaughton, B. R.; Bucholtz, K. M.; Camaano-Moure, A.; Miller, B. L., Self-Selection in Olefin Cross-Metathesis: The Effect of Remote Functionality. *Organic Letters* **2005,** 7, (4), 733-736.

(7S,10S,13S)-13-Amino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester hydrogen chloride salt (5.7)

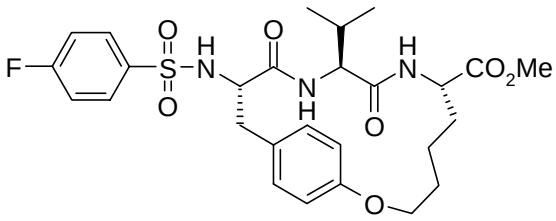


N-BOC protected compound **4.18** (1.00g, 1.97 mmol) was reacted using General Procedure I1 to afford a white solid, 0.873g, 100%

^{1}H NMR ppm (500 in $(CD_3)_2SO$) 8.51 (2H, bs, N**H$_2$**), 8.16 (1H, d J=7.2Hz, N**H** Val), 7.90 (1H, d J=6.4Hz, N**H** Gly), 6.98 (2H, d J=6.8Hz, Ar-**H)** 6.75 (2H, d J=6.8Hz, Ar-**H**), 4.31-4.36 (2H, m, OC**H$_2$**$CH_2CH_2CH_2$), 4.18-4.25 (1H, m, C**H**CO_2CH_3), 4.03-4.08 (1H, m, C**H**CH_2Ph) 3.84 (1H, dd J=5.2Hz, J=7.2Hz, C**H**$CH(CH_3)_2$), 3.60 (3H, s, $CHCO_2C$**H$_3$**), 3.08 (1H, dd J=5.6Hz, J=11.2Hz, CHC**H$_2$**Ph), 2.60 (1H, dd J=11.2Hz, J=11.2Hz, CHC**H$_2$**Ph), 1.96-2.01 (1H, m, CHC**H**$(CH_3)_2$), 1.76-1.83 (1H, m, $OCH_2CH_2CH_2C$**H$_2$**), 1.65-1.74 (1H, m, OCH_2C**H$_2$**CH_2CH_2), 1.48-1.57 (2H, m, $OCH_2CH_2CH_2C$**H$_2$** and OCH_2C**H$_2$**CH_2CH_2), 1.22-1.36 (2H, m, OCH_2CH_2C**H$_2$**CH_2), 0.84 (3H, d J=6.4Hz, $CHCH(C$**H$_3$**$)_2$), 0.77 (3H, d J=6.4Hz, $CHCH(C$**H$_3$**$)_2$)

LRMS (ES) 406.3 (MH^+). $C_{21}H_{31}N_3O_5$ requires 406.2

(7S,10S,13S)-13-(4-Fluoro-benzenesulfonylamino)-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (5.8)

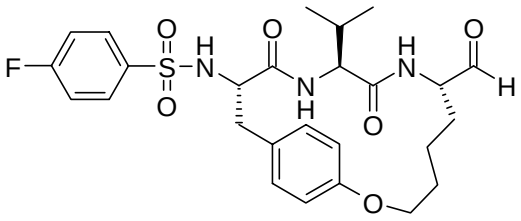


Amine **5.7** (0.200g, 0.452 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield a brown solid, 0.0920g, 36%. R_f = 0.09 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in (CD_3OD) 7.51-7.72 (2H, m, Ar-**H** (4F-Ph)), 7.25-7.29 (1H, m, Ar-**H** (4F-Ph)), 6.87 (2H, d J=8.2Hz, Ar-**H** (Tyr)), 6.74 (2H, d J=8.2Hz, Ar-**H** (Tyr)), 4.24-4.58 (2H, m, OC**H$_2$**$CH_2CH_2CH_2$ and C**H**CO_2CH_3) 3.94-4.27 (3H, m, OC**H$_2$**$CH_2CH_2CH_2$ and C**H**CH_2Ph and C**H**$CH(CH_3)_2$), 3.69 (3H, s, $CHCO_2C$**H$_3$**), 2.95-3.05 (1H, m, CHC**H$_2$**Ph), 2.67 (1H, dd J=9.4Hz, J=9.4Hz, CHC**H$_2$**Ph), 1.97-2.03 (1H, m, CHC**H**$(CH_3)_2$), 1.62-1.83 (2H, m, $OCH_2CH_2CH_2C$**H$_2$** and OCH_2C**H$_2$**CH_2CH_2), 1.44-1.58 (2H, m, $OCH_2CH_2CH_2C$**H$_2$** and OCH_2C**H$_2$**CH_2CH_2), 1.20-1.33 (2H, m, OCH_2CH_2C**H$_2$**CH_2), 0.74 (3H, d J=6.9Hz, $CHCH(C$**H$_3$**$)_2$), 0.63 (3H, d J=6.9Hz, $CHCH(C$**H$_3$**$)_2$)

HRMS (ES) 564.2173 (MH^+). $C_{27}H_{34}FN_3O_7S$ requires 564.2180

4-Fluoro-N-((7S,10S,13S)-7-formyl-10-isopropyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-benzenesulfonamide (5.10)

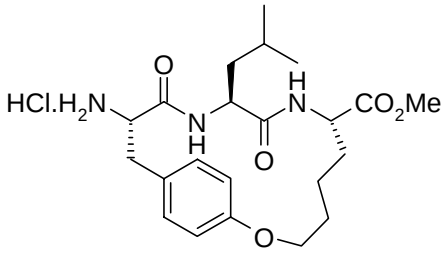


Methyl ester **5.8** (0.100g, 0.177 mmol) was reduced using General Procedure R. The crude material was purified by flash chromatography on silica using EtOAc to yield a brown solid, 0.0161g, 17%. R_f = 0.67 (EtOAc).

^{1}H NMR ppm ((500 MHz in $(CD_3)_2SO$) 9.29 (1H, s, C**H**O), 8.13 (1H, d J=8.2HZ, N**H** Val), 7.91 (1H, d J=6.9Hz, N**H** Gly), 7.48-7.55 (2H, m, Ar-**H** (4F-Ph)), 7.31-7.37 (2H, m, Ar-**H** (4F-Ph)), 6.97 (2H, d J=7.6Hz, Ar-**H** (Tyr)), 6.89 (2H, d J=7.6Hz, Ar-**H** (Tyr)), 6.66 (1H, d J=7.1Hz, N**H**), 4.40-4.49 (3H, m, OC**H$_2$**$CH_2CH_2CH_2$ and C**H**CO_2CH_3), 4.09-4.30 (2H, m, C**H**CH_2Ph and C**H**$CH(CH_3)_2$), 3.01-3.07 (1H, m, CHC**H$_2$**Ph), 2.64 (1H, dd J=11.1Hz, J=11.1Hz, CHC**H$_2$**Ph), 1.99-2.06 (1H, m, CHC**H**$(CH_3)_2$), 1.40-1.87 (4H, m, $OCH_2CH_2CH_2C$**H$_2$** and OCH_2C**H$_2$**CH_2CH_2 and $OCH_2CH_2CH_2C$**H$_2$** and OCH_2C**H$_2$**CH_2CH_2), 1.21-1.38 (2H, m, OCH_2CH_2C**H$_2$**CH_2), 0.59 (3H, d J=6.8 Hz, $CHCH(C$**H$_3$**$)_2$), 0.55 (3H, d J=6.8 Hz, $CHCH(C$**H$_3$**$)_2$)

HRMS (ES) 534.2078 (MH^+). $C_{26}H_{32}FN_3O_6S$ requires 534.2074

(7S,10S,13S)-13-Amino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1-(18),15(19),16-triene-7-carboxylic acid methyl ester hydrogen chloride salt (5.11)

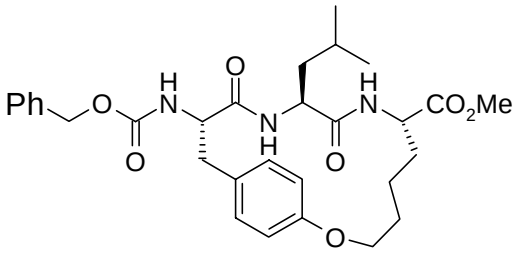


N-BOC protected compound **4.49** (0.340g, 0.654 mmol) was reacted using General Procedure I1 to afford a brown solid, 0.232g, 100%

^{1}H-NMR (500 MHz in $(CD_3)_2SO$) 8.46 (2H, bs, N**H$_2$**), 8.32 (1H, d J=8.7Hz N**H** Leu), 7.95 (1H, d J=7.8Hz, N**H** Gly), 6.80 (2H, d, J=8.2Hz, Ar-**H**), 7.02 (2H, d, J=8.2Hz, Ar-**H**) , 4.22-4.40 (3H, m, C**H**CH_2Ph and C**H**$CH_2CH(CH_3)_2$ and OC**H$_2$**$CH_2CH_2CH_2$), 3.95-4.08 (2H, C**H**CO_2CH_3 and OC**H$_2$**$CH_2CH_2CH_2$), 3.56 (3H, s, CO_2C**H$_3$**), 3.12 (1H, dd, J=5.9Hz, J=12.7Hz, CHC**H$_2$**Ph), 2.62 (1H, dd J=12.7Hz, J=12.7Hz, CHC**H$_2$**Ph), 1.60-1.75 (4H, m, OCH_2CH_2C**H$_2$**CH_2 and $OCH_2CH_2CH_2C$**H$_2$**), 1.49-1.58 (2H, m, CHC**H$_2$**$CH(CH_3)_2$) 1.44-1.48 (1H, m, $CHCH_2C$**H**$(CH_3)_2$), 1.22-1.41 (2H, m, OCH_2C**H$_2$**CH_2CH_2), 0.85 (3H, d J=7.3Hz, $CHCH_2CH(C$**H$_3$**$)_2$), 0.84 (3H, d J=7.3Hz, $CHCH_2CH(C$**H$_3$**$)_2$)

HRMS (ES) 419.2541 (MH^+). $C_{23}H_{34}N_2O_5$ requires 419.2546

(7S,10S,13S)-13-Benzyloxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (5.12)



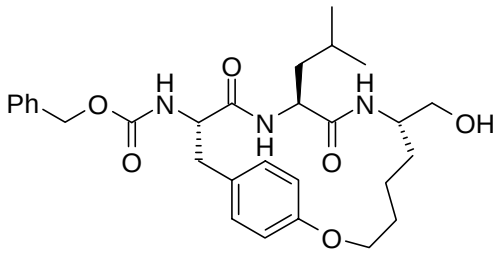
Amine **5.11** (4.00g, 8.78 mmol) was reacted with benzyl chloroformate using General Procedure H1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc/DCM to yield an off-white solid, 2.10g, 43%. R_f = 0.43 (30% EtOAc/DCM).

^{1}H-NMR (500 MHz in $CDCl_3$) 7.31-7.38 (5H, m, Ar-**H** (CBZ)), 7.06 (2H, d J=7.9Hz, Ar-**H** (Tyr)), 6.79 (2H, d J=7.9Hz, Ar-**H** (Tyr)), 6.13 (1H, d J=6.9Hz, N**H** Gly), 5.79 (1H, d J=7.9Hz, N**H** Leu), 5.53 (1H, d J=8.5Hz, N**H** Tyr), 5.13 (2H, s, OC**H$_2$**Ph), 4.51-4.57 (1H, m, C**H**$CH_2CH(CH_3)_2$), 4.22-4.32 (2H, m, C**H**CH_2Ph and OC**H$_2$**$CH_2CH_2CH_2$), 4.11 (1H, ddd, J=5.9Hz, J=10.1Hz, J=16.4Hz, OC**H$_2$**$CH_2CH_2CH_2$), 3.95 (1H, ddd J=6.5Hz, J=6.9Hz, J=13.0Hz, C**H**CO_2CH_3), 3.73 (3H, s, CO_2C**H$_3$**), 3.14 (1H, dd J=5.7Hz, J=12.8Hz, CHC**H$_2$**Ph), 2.67 (1H, dd, J=12.8Hz, J=12.8Hz, CHC**H$_2$**Ph), 1.86-1.95 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.74-1.83 (2H, m, OCH_2C**H$_2$**CH_2CH_2), 1.22-1.58 (6H, m, CHC**H$_2$**$CH(CH_3)_2$ and OCH_2CH_2C**H$_2$**CH_2 and $OCH_2CH_2CH_2$C**H$_2$**), 0.88 (3H, d J=6.0Hz, $CHCH_2CH$(C**H$_3$**)$_2$), 0.87 (3H, d J=6.0Hz, $CHCH_2CH$(C**H$_3$**)$_2$).

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.5, 170.8, 169.7, 157.1, 155.5, 136.3, 130.1, 128.5, 128.2, 128.1, 127.9, 115.7, 66.8, 66.7, 57.1, 52.5, 51.7, 51.2, 43.3, 39.0, 31.5, 28.0, 24.5, 22.9, 22.4, 21.2

HRMS (ES) 554.2859 (MH^+). $C_{30}H_{39}N_3O_7$ requires 554.2866

(7S,10S,13S)-7-Hydroxymethyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-13-yl)carbamic acid benzyl ester (5.13)



Methyl ester **5.12** (2.30g, 4.15 mmol) was reduced using General Procedure S to afford an off-white solid, 1.81g, 83%.

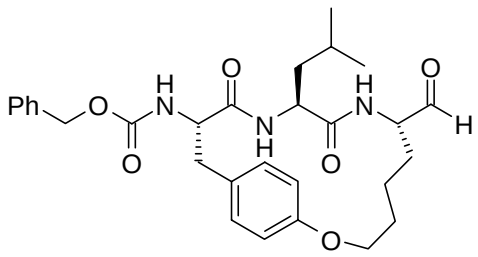
m.p 238-240^{o}C

^{1}H-NMR (500 MHz in CD_3OD) 7.68 (1H, d J=9.1Hz, N**H** Gly), 7.22-7.36 (6H, m, Ar-**H** (CBZ) and N**H** Leu), 7.06 (2H, d, J=7.9Hz, Ar-**H** Tyr), 6.77 (2H, d, J=7.9Hz, Ar-**H** Tyr), 5.10 (1H, d J=12.5Hz, OC**H$_2$**Ph), 5.05 (1H, d J=12.1 Hz, OC**H$_2$**Ph), 4.26-4.34 (2H, m, C**H**CH_2Ph and OC**H$_2$**$CH_2CH_2CH_2$), 4.06-4.12 (1H, m, OC**H$_2$**$CH_2CH_2CH_2$), 3.96-4.02 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.74-3.86 (1H, m, C**H**CH_2OH), 3.30-3.33 (2H, m, C**H$_2$**OH), 2.98 (1H, dd J=5.4Hz, J=12.7Hz, CHC**H$_2$**Ph), 2.67 (1H, dd, J=12.4Hz, J=12.7Hz, CHC**H$_2$**Ph), 1.75-1.84 (2H, m, OCH_2C**H$_2$**CH_2CH_2), 1.46-1.56 (3H, m, CHC**H$_2$**$CH(CH_3)_2$ and $CHCH_2$C**H**$(CH_3)_2$), 1.22-1.44 (4H, m, OCH_2CH_2C**H$_2$**CH_2 and $OCH_2CH_2CH_2$C**H$_2$**), 0.84 (3H, d J=8.8Hz, $CHCH_2CH(CH_3)_2$), 0.83 (3H, d J=8.8Hz, $CHCH_2CH(CH_3)_2$)

HRMS (ES) 526.2920 (MH^+). $C_{29}H_{39}N_3O_6$ requires 526.2917

Microanalysis. C, 64.08; H, 7.22; N, 7.23. $C_{29}H_{39}N_3O_6.H_2O$ requires C, 64.07; H, 7.60; N, 7.73

(7S,10S,13S)-7-Formyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)carbamic acid benzyl ester (5.14)



Alcohol **5.13** (1.71g, 3.25 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield an off white solid, 0.720g, 42%. R_f = 0.41 (2/1 (EtOAc / (50/70) Pet ether)).
m.p 223-225^{o}C

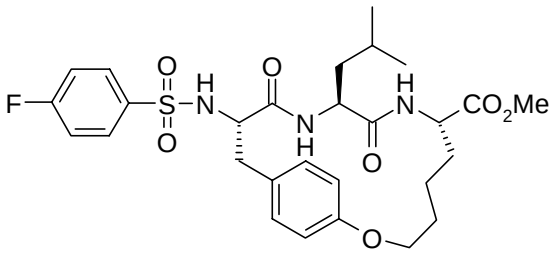
^{1}H-NMR (500 MHz in $(CD_3)_2SO$) 9.33 (1H, s, C**H**O), 8.05 (1H, d J=8.1Hz, N**H** Leu), 7.55 (1H, d J=6.8Hz, N**H** Tyr), 7.30-7.37 (5H, m, Ar-**H** CBZ), 7.16 (1H, d, J=8.1Hz, N**H** Gly), 7.02 (2H, d, J=8.0Hz, Ar-**H** Tyr), 6.77 (2H,d J=8.0Hz, Ar-**H** Tyr), 5.06 (1H, d J=12.3Hz, OC**H$_2$**Ph), 5.01 (1H, d J=12.3Hz, OC**H$_2$**Ph), 4.31-4.36 (2H, m, C**H**CH_2Ph and OC**H$_2$**$CH_2CH_2CH_2$), 4.18-4.25 (1H, m, C**H**$CH_2CH(CH_3)_2$), 4.00-4.07 (2H, m, C**H**CHO and OC**H$_2$**$CH_2CH_2CH_2$), 2.86 (1H, dd J=5.6Hz, J=12.8Hz CHC**H$_2$**Ph), 2.63 (1H, dd J=12.8Hz, J=12.8Hz, CHC**H$_2$**Ph)), 1.70-1.77 (2H, m, OCH_2C**H$_2$**CH_2CH_2), 1.46-1.52 (1H, m, $CHCH_2$C**H**$(CH_3)_2$), 1.22-1.39 (6H, m, CHC**H$_2$**$CH(CH_3)_2$ and $OCH_2CH_2CH_2$C**H$_2$** and OCH_2CH_2C**H$_2$**CH_2), 0.80-0.83 (6H, m, $CHCH_2CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz $CDCl_3$). 199.1, 171.1, 155.9, 137.7, 136.4, 129.3, 128.6, 128.5, 128.0, 127.9, 126.7, 66.6, 57.2, 50.2, 40.2, 38.6, 37.7, 24.8, 23.0, 21.8

HRMS (ES) 524.2762 (MH^+). $C_{29}H_{37}N_3O_6$ requires 524.2760

Microanalysis. C, 64.08; H, 6.95; N, 7.62. $C_{29}H_{37}N_3O_6.H_2O$ requires C, 64.31; H, 7.26; N, 7.76

(7S,10S,13S)-13-(4-Fluoro-benzenesulfonylamino)-10-isobutyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (5.15)

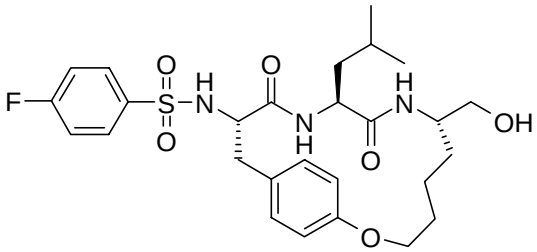


Amine **5.11** (0.232g, 0.508 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield a brown solid, 0.0190g, 7%. R_f = 0.13 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H-NMR (500 MHz 500 MHz in $(CD_3)_2SO$) 8.11-8.15 (2H, m, N**H** Tyr and N**H** Gly), 7.91-7.95 (1H, m, N**H** Leu), 7.92-7.96 (2H, m, Ar-**H** (4-F-Ph)), 7.33-7.37 (2H, m, Ar-**H** (4-F-Ph)), 6.92-6.97 (2H, m, Ar-**H** (Tyr)), 6.70 (2H, d J=7.9Hz, Ar-**H** (Tyr)), 4.28-4.38 (2H, m, C**H**COCH$_3$ and OC**H$_2$**CH$_2$CH$_2$CH$_2$), 4.11-4.21 (2H, m, C**H**CH$_2$Ph and OC**H$_2$**CH$_2$CH$_2$CH$_2$), 3.95-4.04 (1H, m, C**H**CH$_2$CH(CH$_3$)$_2$), 3.52 (3H, s, CO$_2$C**H$_3$**), 2.69-2.75 (1H, m, CHC**H$_2$**Ph), 2.54 (1H, dd J=7.1Hz, J=12.0Hz, CHC**H$_2$**Ph), 1.73-1.78 (2H, m, OCH$_2$C**H$_2$**CH$_2$CH$_2$), 1.20-1.70 (7H, m, OCH$_2$CH$_2$C**H$_2$**CH$_2$ and OCH$_2$CH$_2$CH$_2$C**H$_2$** and CHC**H$_2$**CH(CH$_3$)$_2$ and CHCH$_2$C**H**(CH$_3$)$_2$), 0.73 (3H, d J=6.3Hz, CHCH$_2$CH(C**H$_3$**)$_2$), 0.72 (3H, d J=6.3Hz, CHCH$_2$CH(C**H$_3$**)$_2$).

HRMS (ES) 578.2337 (MH$^+$). $C_{28}H_{36}FN_3O_7S$ requires 578.2336

4-Fluoro-N-((7S,10S,13S)-7-hydroxymethyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-13-yl)benzenesulfonamide (5.16)



Methyl ester **5.15** (0.677g, 1.17 mmol) was reduced using General Procedure S to afford a brown solid, 0.550g, 87%.

m.p 256-258^{o}C

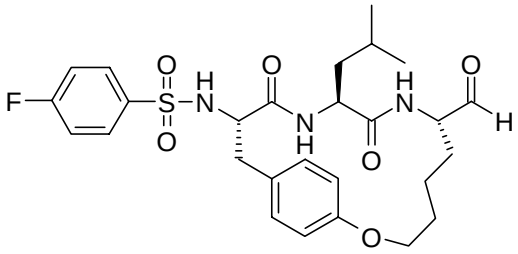
^{1}H-NMR (500 MHz in CD_3OD) 7.95-7.99 (2H, m, Ar-**H** (4-F-Ph)), 7.67 (1H, d J=8.8Hz, N**H** Leu), 7.25-7.28 (2H, m, Ar-**H** (4-F-Ph)), 7.17 (1H, d J=7.5Hz, N**H** Gly), 6.97 (2H, d J=8.0Hz, Ar-**H** (Tyr)), 6.76 (2H, d J=8.0Hz, Ar-**H** (Tyr)), 4.29 (1H, m, OC**H$_2$**CH$_2$CH$_2$CH$_2$), 4.08 (2H, m, C**H**CH$_2$Ph and OC**H$_2$**CH$_2$CH$_2$CH$_2$), 3.85-3.87 (2H, m, C**H**CH$_2$OH and C**H**CH$_2$CH(CH$_3$)$_2$), 3.24-3.33 (2H, m, C**H$_2$**OH), 2.88 (1H, dd J=5.5Hz, J=12.3Hz,

CHC**H$_2$**Ph), 2.69 (1H, dd J=12.3Hz, J=12.3Hz, CHC**H$_2$**Ph), 1.72-1.81 (2H m, OCH$_2$C**H$_2$**CH$_2$CH$_2$), 1.22-1.68 (7H, m, OCH$_2$CH$_2$C**H$_2$**CH$_2$ and OCH$_2$CH$_2$CH$_2$C**H$_2$** and CHC**H$_2$**CH(CH$_3$)$_2$ and CHCH$_2$C**H**(CH$_3$)$_2$), 0.78 (3H, d J=6.4Hz, CHCH$_2$CH(C**H$_3$**)$_2$), 0.76 (3H, d, J=6.4Hz, CHCH$_2$CH(C**H$_3$**)$_2$)

^{13}C NMR ppm (75 MHz in CD$_3$OD). 171.6, 169.7, 166.6, 163.3, 157.1, 130.0, 129.8, 129.6, 127.7, 116.1, 115.8, 66.7, 64.4, 57.9, 51.8, 50.1, 43.2, 39.0, 29.7, 28.3, 24.2, 22.2, 22.1, 21.4.

HRMS (ES) 550.2369 (MH$^+$). $C_{27}H_{36}FN_3O_6S$ requires 550.2387

4-Fluoro-N-((7S,10S,13S)-7-formyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-13-yl)benzenesulfonamide (5.17)



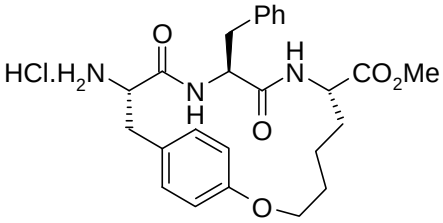
Alcohol **5.16** (0.570g, 1.04 mmol) was oxidised using General Procedure L. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield an off white solid, 0.238g, 42%. R_f = 0.41 (2/1 (EtOAc / (50/70) Pet ether)).
m.p 243-245^{o}C

^{1}H-NMR (500 MHz in (CD$_3$)$_2$SO) 9.29 (1H, s,C**H**O), 8.15 (1H, d J=8.9Hz, N**H** Tyr), 8.04 (1H, d J=8.3Hz, N**H** Leu), 7.93-7.97 (2H, m, Ar-**H** (4-F-Ph)), 7.49 (1H, d J=7.8Hz, N**H** Gly), 7.33-7.38 (2H, m, Ar-**H** (4-F-Ph)), 6.95 (2H, d J=7.6Hz, Ar-**H** (Tyr)), 6.73 (2H, d J=7.6Hz, Ar-**H** (Tyr)), 4.22-4.37 (2H, m, C**H**CH$_2$Ph and OC**H$_2$**CH$_2$CH$_2$CH$_2$), 4.17-4.24 (1H, m, C**H**CH$_2$CH(CH$_3$)$_2$), 3.97-4.04 (1H, m, OC**H$_2$**CH$_2$CH$_2$CH$_2$), 3.80-3.87 (1H, m, C**H**CHO), 2.72 (1H, dd J=5.2Hz, J=12.7Hz, CHC**H$_2$**Ph), 2.54-2.60 (1H, m, CHC**H$_2$**Ph), 1.32-1.74 (7H, m, OCH$_2$CH$_2$C**H$_2$**CH$_2$ and OCH$_2$CH$_2$CH$_2$C**H$_2$** and CHC**H$_2$**CH(CH$_3$)$_2$ and CHCH$_2$C**H**(CH$_3$)$_2$) 0.70-0.75 (6H, m, CHCH$_2$CH(C**H$_3$**)$_2$).

^{13}C NMR ppm (75 MHz in (CD$_3$)$_2$SO). 201.0, 171.0, 168.4, 155.9, 130.3, 129.8, 129.7, 127.8, 116.1, 115.8, 115.5, 66.0, 56.5, 56.3, 50.5, 43.2, 26.8, 26.3, 23.8, 23.1, 22.3, 21.4.

HRMS (ES) 548.2226 (MH$^+$). $C_{27}H_{34}FN_3O_6S$ requires 548.2230

(7S,10S,13S)-13-Amino-10-benzyl-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester hydrogen chloride salt (5.18)

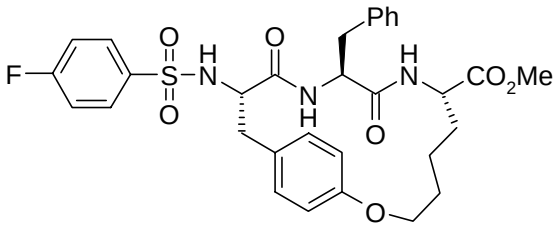


N-BOC protected compound **4.54** (0.530g, 0.958 mmol) was reacted using General Procedure I1 to afford a brown solid, 0.469g, 100%

^{1}H NMR ppm (500 in (CD_3)OD) 7.08-7.20 (5H, m, Ar-**H**), 7.02 (2H, J=8.7Hz, Ar-**H**), 6.80 (2H, d J=8.7Hz, Ar-**H**), 4.28-4.40 (2H, m, OC**H$_2$**$CH_2CH_2CH_2$), 4.14 (1H, dd J=6.1Hz, J=11.3Hz, C**H**CO_2CH_3), 4.02-4.10 (1H, m, C**H**CH_2Ph (Tyr) and C**H**CH_2Ph(Phe)), 3.63 (3H, s, $CHCO_2C$**H$_3$**), 3.19 (1H, dd J=6.1Hz, J=12.1Hz, CHC**H$_2$**Ph (Tyr)), 2.96-3.00 (2H, m, CHC**H$_2$**Ph (Tyr) and CHC**H$_2$**Ph (Phe)), 2.77 (1H, dd J=12.4, J=12.4Hz, CHC**H$_2$**Ph (Phe)), 2.63 (1H, dd J=12.1Hz, J=12.1Hz, CHC**H$_2$**Ph (Tyr)),)), 1.70-1.80 (1H, m, $OCH_2CH_2CH_2C$**H$_2$**), 1.54-1.62 (1H, m, OCH_2C**H$_2$**CH_2CH_2), 1.24-1.39 (4H, m, $OCH_2CH_2CH_2C$**H$_2$** and OCH_2CH_2C**H$_2$**CH_2 and OCH_2C**H$_2$**CH_2CH_2)

LRMS (ES) 454.2 (MH^+). $C_{25}H_{31}N_3O_5$ requires 454.2

(7S,10S,13S)-10-Benzyl-13-(4-fluoro-benzenesulfonylamino)-9,12-dioxo-2-oxa-8,11-diazabicyclo[13.2.2]-nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (5.19)

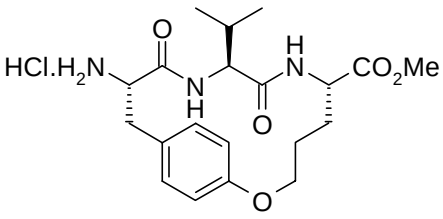


Amine **5.18** (0.200g, 0.408 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield a brown solid, 0.0450g, 18%. R_f = 0.11 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in (CD_3OD) 7.52-7.63 (2H, m, Ar-**H** (4F-Ph)), 7.26-7.31 (2H, m, Ar-**H** (4F-Ph)), 7.06-7.22 (5H, m, Ar-**H**), 6.85 (2H, d J=8.0Hz, Ar-**H** (Tyr)), 6.76 (2H, d J=8.0Hz, Ar-**H** (Tyr)), 4.24-4.44 (2H, m, OC**H$_2$**$CH_2CH_2CH_2$ and C**H**CO_2CH_3) 3.98-4.21 (3H, m, OC**H$_2$**$CH_2CH_2CH_2$ and C**H**CH_2Ph (Tyr) and C**H**CH_2Ph(Phe)), 3.71 (3H, s, $CHCO_2C$**H$_3$**), 3.14 (1H, dd J=6.2Hz, J=12.1Hz, CHC**H$_2$**Ph (Tyr)), 2.94-3.02 (2H, m, CHC**H$_2$**Ph (Tyr) and CHC**H$_2$**Ph (Phe)), 2.74 (1H, dd J=12.5, J=12.5Hz, CHC**H$_2$**Ph (Phe)), 2.61 (1H, dd J=12.1Hz, J=12.1Hz, CHC**H$_2$**Ph (Tyr)), 1.71-1.82 (1H, m, $OCH_2CH_2CH_2C$**H$_2$**), 1.54-1.66 (1H, m, OCH_2C**H$_2$**CH_2CH_2), 1.20-1.44 (4H, m, $OCH_2CH_2CH_2C$**H$_2$** and OCH_2CH_2C**H$_2$**CH_2 and OCH_2C**H$_2$**CH_2CH_2)

LRMS (ES) 612.2 (MH^+). $C_{31}H_{34}FN_3O_7S$ requires 612.2

(6S,9S,12S)-12-Amino-9-isopropyl-8,11-dioxo-2-oxa-7,10-diaza-bicyclo[12.2.2]octadeca-1(17),14(18),15-triene-6-carboxylic acid methyl ester hydrochloride (5.22)

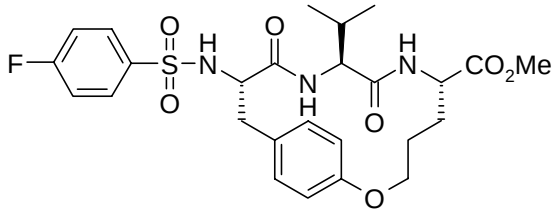


N-BOC protected compound **4.30** (0.250g, 0.509 mmol) was reacted using General Procedure I1 to afford a brown solid, 0.217g, 100%

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 8.38 (2H, bs, N**H$_2$**), 8.13 (1H, d J=7.1Hz, N**H** Gly), 7.79 (1H, d J=8.7Hz, N**H** Val), 7.16 (2H, d J=8.1Hz, Ar-**H**), 6.71 (2H, d, J=8.1Hz, Ar-**H**), 4.29-4.41 (2H, m, OC**H$_2$**CH_2CH_2), 4.22-4.28 (1H, m, C**H**CO_2CH_3), 4.06-4.17 (1H, m, C**H**CH_2Ph), 3.80 (1H, dd J=8.2Hz, J=8.7Hz, C**H**$CH(CH_3)_2$), 3.57 (3H, s, $CHCO_2C$**H$_3$**), 3.14 (1H, dd J=6.0Hz, J=12.1Hz, CHC**H$_2$**Ph), 2.57 (1H, dd J=12.1Hz, J=12.1Hz, CHC**H$_2$**Ph), 1.91-2.06 (1H, m, CHC**H**$(CH_3)_2$), 1.78-1.90 (1H, m, OCH_2CH_2C**H$_2$**), 1.53-1.74 (2H, m, OCH_2CH_2C**H$_2$** and OCH_2C**H$_2$**CH_2) 1.21-1.42 (1H, m, OCH_2C**H$_2$**CH_2), 0.80 (3H, d J=6.9Hz, $CHCH(C$**H$_3$**$)_2$), 0.78 (3H, d J=6.9Hz, $CHCH(C$**H$_3$**$)_2$)

HRMS (ES) 392.2179 (MH^+). $C_{20}H_{29}N_3O_5$ requires 392.2185

(6S,9S,12S)-12-(4-Fluoro-benzenesulfonylamino)-9-isopropyl-8,11-dioxo-2-oxa-7,10-diazabicyclo[12.2.2]-octadeca-1(17),14(18),15-triene-6-carboxylic acid methyl ester (5.23)

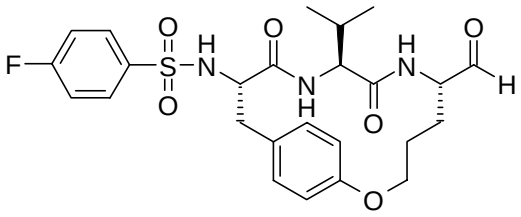


Amine **5.22** (0.170g, 0.397 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield a brown solid, 0.0350g, 16%. R_f = 0.17 (4/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 7.56-7.64 (2H, m, Ar-**H** (4-F-Ph)), 7.18-7.35 (2H, m, Ar-**H** (4-F-Ph)), 6.86 (2H, d, J=8.2Hz, Ar-**H**), 6.73 (2H, d J=8.2Hz, Ar-**H**), 4.16-4.27 (2H, m, OC**H$_2$**CH_2CH_2), 4.06-4.18 (1H, m, C**H**CO_2CH_3), 3.91-4.05 (1H, m, C**H**CH_2Ph), 3.82-3.91 (1H, m, C**H**$CH(CH_3)_2$), 3.70 (3H, s, $CHCO_2C$**H$_3$**), 2.94-3.02 (1H, m, CHC**H$_2$**Ph), 2.91 (1H, dd J=6.9Hz, J=13.1Hz, CHC**H$_2$**Ph), 1.95-2.06 (1H, m, CHC**H**$(CH_3)_2$), 1.70-1.84 (1H, m, OCH_2CH_2C**H$_2$**), 1.24-1.69 (3H, m, OCH_2CH_2C**H$_2$** and OCH_2C**H$_2$**CH_2), 0.87 (3H, d J=6.9Hz, $CHCH(C$**H$_3$**$)_2$), 0.84 (3H, d J=6.9Hz, $CHCH(C$**H$_3$**$)_2$)

HRMS (ES) 550.2198 (MH^+). $C_{26}H_{32}FN_3O_7S$ requires 550.2023

4-Fluoro-N-((6S,9S,12S)-6-formyl-9-isopropyl-8,11-dioxo-2-oxa-7,10-diaza-bicyclo-[12.2.2]octadeca-1(17),14(18),15-trien-12-yl)benzenesulfonamide (5.25)

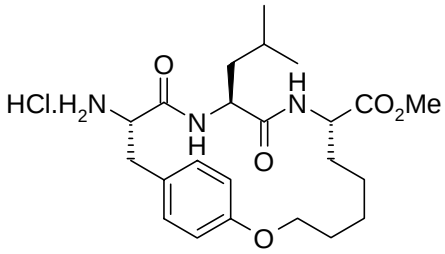


Methyl ester **5.23** (0.0350g, 0.0637 mmol) was reduced using General Procedure R. The crude material was purified by flash chromatography on silica using EtOAc to yield a brown solid, 0.00180g, 5%. R_f = 0.34 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 7.68-7.71 (2H, m, Ar-**H** (4-F-Ph)),, 7.58-7.61 (2H, m, Ar-**H** (4-F-Ph)), 6.79 (2H, d J=8.0Hz, Ar-**H** (Tyr)), 6.48 (2H, d J=8.0Hz, Ar-**H** (Tyr)), 4.16-4.27 (4H, m, OC**H$_2$**CH_2CH_2 and C**H**CO_2CH_3 and C**H**CH_2Ph), 3.84-3.90 (1H, m, C**H**$CH(CH_3)_2$), 2.86-3.01 (2H, m, CHC**H$_2$**Ph), 1.63-1.71 (1H, m, CHC**H**$(CH_3)_2$), 1.28-1.41 (4H, m, OCH_2CH_2C**H$_2$** and OCH_2C**H$_2$**CH_2), 0.93 (3H, d J=7.5Hz, $CHCH(C$**H$_3$**$)_2$), 0.90 (3H, d J=7.5Hz, $CHCH(C$**H$_3$**$)_2$)

HRMS (ES) 520.1911 (MH^+). $C_{25}H_{30}FN_3O_6S$ requires 520.1917

(8S,11S,14S)-14-Amino-11-isobutyl-10,13-dioxo-2-oxa-9,12-diazabicyclo[14.2.2]icosa-1(19),16(20),17-triene-8-carboxylic acid methyl ester hydrochloride (5.26)



N-BOC protected compound **4.42** (0.600g, 1.12 mmol) was reacted using General Procedure I1 to afford an off white solid, 0.528g, 100%

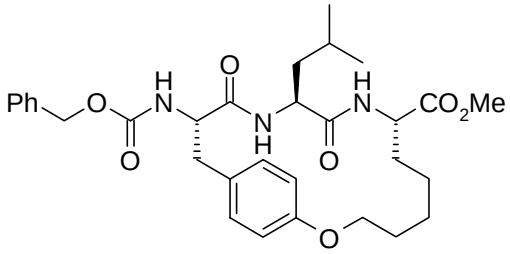
^{1}H NMR ppm (500 MHz in CD_3OD) 7.04 (2H, d J=8.1Hz, Ar-**H**), 6.79 (2H, d J=8.1Hz, Ar-**H**), 4.24-4.27 (1H, m, C**H**CO_2CH_3), 4.11-4.20 (2H, m, OC**H$_2$**$CH_2CH_2CH_2CH_2$ and C**H**$CH_2CH(CH_3)_2$), 4.07 (1H, dd J=4.7Hz, J=10.9Hz, C**H**CH_2Ph), 3.96-4.01 (1H, m, OC**H$_2$**$CH_2CH_2CH_2CH_2$), 3.64 (3H, s, $CHCO_2C$**H$_3$**), 3.16 (1H, dd J=4.7Hz, J=12.5Hz, CHC**H$_2$**Ph), 2.81 (1H, dd J=10.9Hz, J=12.5Hz, CHC**H$_2$**Ph), 1.69-1.79 (2H m, OCH_2C**H$_2$**$CH_2CH_2CH_2$), 1.49-1.61 (3H, m, CHC**H$_2$**$CH(CH_3)_2$ and $CHCH_2C$**H**$(CH_3)_2$), 1.34-1.47 (2H, m, $OCH_2CH_2CH_2CH_2C$**H$_2$**), 1.25-1.33 (4H, m, OCH_2CH_2C**H$_2$**CH_2CH_2 and $OCH_2CH_2CH_2C$**H$_2$**CH_2), 0.90 (3H, d J=6.4 Hz, $CHCH_2CH(C$**H$_3$**$)_2$), 0.88 (3H, d J=6.4 Hz, $CHCH_2CH(C$**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in CD_3OD) 172.7, 171.5, 166.8, 158.1, 130.0, 125.7, 115.1, 66.4, 54.1, 51.9, 51.4, 51.3, 42.6, 36.3, 31.1, 27.3, 24.1, 24.0, 23.8, 22.0, 21.6

HRMS (ES) 456.2444 (MNa^+). $C_{23}H_{35}N_3O_5$ requires 456.2474

FTIR (KBr) 3327, 2931, 862, 1743, 1681, 1654, 1541, 1508

(8S,11S,14S)-14-Benzyloxycarbonylamino-11-isobutyl-10,13-dioxo-2-oxa-9,12-diaza-bicyclo[14.2.2]icosa-1(19),16(20),17-triene-8-carboxylic acid methyl ester (5.27)

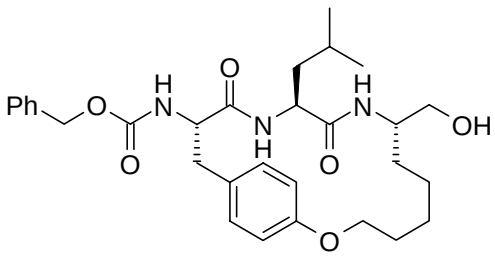


Amine **5.26** (0.550g, 1.17 mmol) was reacted with benzyl chloroformate using General Procedure H1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc/DCM to yield an off-white solid, 0.460g, 69%. R_f = 0.32 (30% EtOAc/DCM).

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 8.09 (1H, d J=7.5Hz, N**H** Leu), 7.42 (1H, d J=6.9Hz, N**H** Tyr), 7.30-7.36 (6H, m, N**H** Gly and Ar-**H** (CBZ)), 7.02 (2H, d J=8.3Hz, Ar-**H** Tyr), 6.71 (2H, d J=8.3Hz, Ar-**H** Tyr), 5.06 (1H, d J=12.7 Hz, OC**H$_2$**Ph), 5.01 (1H, d J=12.7Hz, OC**H$_2$**Ph), 4.32-4.38 (1H, m, C**H**CH_2Ph), 4.06-4.15 (3H, m, C**H**CO_2CH_3 and C**H**$CH_2CH(CH_3)_2$ and OC**H$_2$**$CH_2CH_2CH_2CH_2$), 3.98 (1H, td J=5.1Hz, J=10.0Hz, OC**H$_2$**$CH_2CH_2CH_2CH_2$), 3.57 (3H, s, $CHCO_2C$**H$_3$**), 2.86 (1H, dd J=5.2Hz, J=12.1Hz, CHC**H$_2$**Ph), 2.68 (1H, dd J=12.1Hz, J=12.1Hz, CHC**H$_2$**Ph), 1.61-1.68 (2H, m, OCH_2C**H$_2$**$CH_2CH_2CH_2$), 1.54-1.60 (1H, m, $OCH_2CH_2CH_2CH_2C$**H$_2$**), 1.40-1.52 (3H, m, CHC**H$_2$**$CH(CH_3)_2$ and $CHCH_2C$**H**$(CH_3)_2$), 1.16-1.36 (5H, m, $OCH_2CH_2CH_2CH_2C$**H$_2$** and $OCH_2CH_2CH_2C$**H$_2$**CH_2 and OCH_2CH_2C**H$_2$**$CH_2CH_2CH_2$), 0.82 (3H, d J=6.5 Hz, $CHCH_2CH(C$**H$_3$**$)_2$), 0.80 (3H, d J=6.5 Hz, $CHCH_2CH(C$**H$_3$**$)_2$)

HRMS (ES) 568.3051 (MH^+). $C_{31}H_{41}N_3O_7$ requires 568.3023

(8S,11S,14S)-8-Hydroxymethyl-11-isobutyl-10,13-dioxo-2-oxa-9,12-diaza-bicyclo-[14.2.2]icosa-1(19),16(20),17-trien-14-yl)carbamic acid benzyl ester (5.28)



Methyl ester **5.27** (0.300g, 0.528 mmol) was reduced using General Procedure S to afford a brown solid, 0.206g, 72%

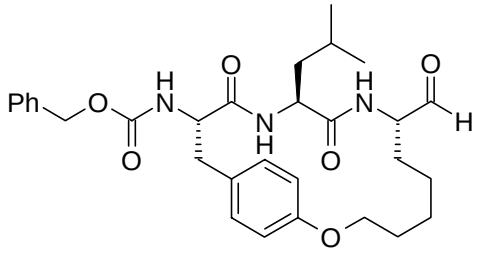
mp. 208-210^{o}C

^{1}H NMR ppm (500 MHz in CD_3OD) 7.57 (1H, d J=9.1Hz, N**H** Gly), 7.35 (1H, d J=7.3Hz, N**H** Leu), 7.23-7.32 (5H, m, Ar-**H** (CBZ)), 7.20 (1H, d J=6.7Hz, N**H** Tyr), 6.97 (2H, d J=8.2Hz, Ar-**H** Tyr), 6.67 (2H, d J=8.2Hz, Ar-**H** Tyr), 5.08 (1H, d J=12.2 Hz, OC**H$_2$**Ph), 5.02 (1H, d J=12.6 Hz, OC**H$_2$**Ph), 4.21 (1H, dd J=6.7Hz, J=12.0Hz, C**H**CH_2Ph), 4.03-4.10 (2H, m, OC**H$_2$**$CH_2CH_2CH_2CH_2$ and C**H**$CH_2CH(CH_3)_2$), 3.90-3.97 (1H, m, OC**H$_2$**$CH_2CH_2CH_2CH_2$), 3.56-3.58 (1H, m, C**H**CH_2OH), 3.28 (2H, d J=5.4Hz, CHC**H$_2$**OH), 2.87 (1H, dd J=5.0Hz, J=12.0Hz, CHC**H$_2$**Ph), 2.68 (1H, dd J=12.0Hz, J=12.0Hz, CHC**H$_2$**Ph), 1.57-1.70 (2H, m, OCH_2C**H$_2$**CH_2CH_2), 1.32-1.47 (4H, m, $OCH_2CH_2CH_2CH_2$C**H$_2$** and CHC**H$_2$**$CH(CH_3)_2$ and $CHCH_2$C**H**$(CH_3)_2$), 1.14 (5H, m, $OCH_2CH_2CH_2CH_2$C**H$_2$** and $OCH_2CH_2CH_2$C**H$_2$**CH_2 and OCH_2CH_2C**H$_2$**CH_2CH_2), 0.76 (6H d J=6.6Hz, $CHCH_2CH$(C**H$_3$**$)_2$)

HRMS (ES) 540.3061 (MH^+). $C_{30}H_{41}N_3O_6$ requires 540.3073

Microanalysis. C, 65.76; H, 7.33; N, 7.29. $C_{30}H_{41}N_3O_6.½H_2O$ requires C, 65.67; H, 7.72; N, 7.66

(8S,11S,14S)-8-Formyl-11-isobutyl-10,13-dioxo-2-oxa-9,12-diaza-bicyclo[14.2.2]icosa-1(19),16(20),17-trien-14-yl)-carbamic acid benzyl ester (5.29)



Alcohol **5.28** (0.300g, 0.554 mmol) was oxidised using General Procedure L to afford an off white solid, 0.269g, 90%.

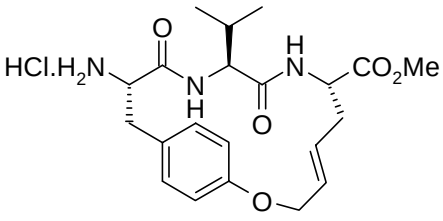
m.p 238-241^{o}C

^{1}H NMR ppm (500 MHz in $CDCl_3$) 9.49 (1H, s, C**H**O), 7.31-7.39 (5H, m, Ar-**H** (CBZ)), 7.07 (2H, d J=7.9Hz, Ar-**H** (Tyr)), 6.76 (2H, d J=7.9Hz, Ar-**H** (Tyr)), 6.23 (1H, d J=7.5Hz, N**H** Leu), 6.09 (1H, d J=6.8Hz, N**H** Gly), 5.64 (1H, d J=8.8Hz, N**H** Tyr), 5.12 (2H, s, OC**H$_2$**Ph), 4.39 (1H, ddd J=5.4Hz, J=6.8Hz, J=7.4Hz, C**H**CHO), 4.26-4.36 (1H, m, C**H**CH_2Ph), 4.07-4.22 (1H, m, OC**H$_2$**$CH_2CH_2CH_2CH_2$), 4.03 (1H, ddd J=5.1Hz, J=7.5Hz, J=10.2Hz, C**H**$CH_2CH(CH_3)_2$), 3.09 (1H, dd J=4.9Hz, J=12.6, CHC**H$_2$**Ph), 2.77 (1H, dd J=11.2Hz, J=12.6Hz, CHC**H$_2$**Ph), 1.70-1.82 (3H, m, $OCH_2CH_2CH_2CH_2$C**H$_2$** and $CHCH_2$C**H**$(CH_3)_2$), 1.39-1.62 (3H, m, $OCH_2CH_2CH_2CH_2$C**H$_2$** and CHC**H$_2$**$CH(CH_3)_2$), 1.14 (5H, m, OCH_2C**H$_2$**$CH_2CH_2CH_2$ and OCH_2CH_2C**H$_2$**CH_2CH_2 and $OCH_2CH_2CH_2$C**H$_2$**CH_2), 0.88 (3H, d J=5.4Hz, $CHCH_2CH$(C**H$_3$**$)_2$), 0.86 (3H, d J=5.4Hz, $CHCH_2CH$(C**H$_3$**$)_2$)

HRMS (ES) 538.2918 (MH^+). $C_{30}H_{39}N_3O_6$ requires 538.2917

Microanalysis. C, 64.10; H, 7.22; N, 7.17. $C_{30}H_{39}N_3O_6.1½H_2O$ requires C, 63.81; H, 7.22; N, 7.44

(E)-(7S,10S,13S)-13-Amino-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (5.34)

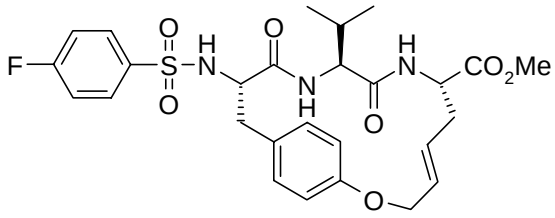


N-BOC protected compound **4.16** (0.900g, 1.79 mmol) was reacted using General Procedure I1 to afford an off white solid, 0.815g, 100%

^{1}H NMR ppm (500 MHz in CD_3OD) 8.29 (1H, d J=9.0Hz, N**H** Gly), 7.58 (1H, d J=7.4Hz, N**H** Val), 7.00 (2H, d J=8.4Hz, Ar-**H**), 6.73 (2H, d J=8.4Hz, Ar-**H**), 5.64 (1H, td J=4.0Hz, J=7.5Hz, $OCH_2C\mathbf{H}CHCH_2$), 5.50-5.56 (1H, m, $OCH_2CHC\mathbf{H}CH_2$), 4.61-4.64 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.53 (1H, ddd, J=3.0Hz, J=9.0Hz, J=12.2Hz, $C\mathbf{H}CO_2CH_3$), 4.12 (1H, dd J=5.8Hz, J=10.4Hz, $C\mathbf{H}CH_2Ph$), 3.93-3.96 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.66 (3H, s, $CO_2C\mathbf{H_3}$), 3.14 (1H, dd J=5.8Hz, J=13.0Hz, $CHC\mathbf{H_2}Ph$), 2.82 (1H, dd J=10.4Hz, J=13.0Hz, $CHC\mathbf{H_2}Ph$), 2.61-2.68 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 2.27 (1H, ddd J=7.9Hz, J=12.2Hz, J=15.0Hz, $OCH_2CHCHC\mathbf{H_2}$), 1.96-2.07 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 0.91 (3H, d J=6.9 Hz, $CHCH(C\mathbf{H_3})_2$), 0.86 (3H, d J=6.9Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 404.2192 (MH^+). $C_{21}H_{29}N_3O_5$ requires 404.2185

(E)-(7S,10S,13S)-13-(4-Fluorobenzenesulfonylamino)-10-isopropyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (5.35)

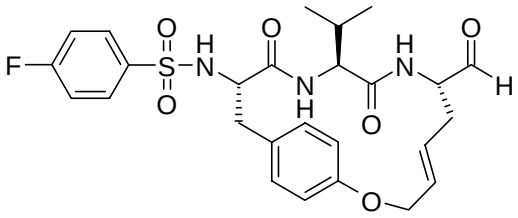


Amine **5.34** (0.810g, 1.78 mmol) was reacted with 4-fluorobenzene sulfonyl chloride using General Procedure M3. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) per ether to yield a brown solid, 0.329g, 33%. R_f = 0.23 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CD_3(SO)$) 8.10-8.13 (2H, m, N**H** Tyr and N**H** Gly), 7.93-7.97 (2H, m, Ar-**H** (4-F-Ph)), 7.50 (1H, d J=8.5Hz, N**H** Val), 7.35-7.40 (2H, m, Ar-**H** (4-F-Ph)), 6.90 (2H, d J=8.2Hz, Ar-**H** (Tyr)), 6.62 (2H, d J=8.2Hz, Ar-**H** (Tyr)) , 5.54-5.59 (1H, m, $OCH_2C\mathbf{H}CHCH_2$), 5.40-5.46 (1H, m, $OCH_2CHC\mathbf{H}CH_2$), 4.55-4.64 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.31-4.36 (2H, m, $C\mathbf{H}CO_2CH_3$ and $C\mathbf{H}CH_2Ph$), 3.75 (1H, dd J=6.0Hz, J=8.5Hz, $C\mathbf{H}CH(CH_3)_2$), 3.58 (3H, s, $CO_2C\mathbf{H_3}$), 2.61-2.67 (2H, m, $CHC\mathbf{H_2}Ph$), 2.46 (1H, ddd J=4.0Hz, J=5.2Hz, J=6.5Hz, $OCH_2CHCHC\mathbf{H_2}$), 2.15-2.22 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 1.52 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 0.61 (3H, d J=6.9 Hz, $CHCH(C\mathbf{H_3})_2$), 0.57 (3H, d J=6.9Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 562.2011 (MH^+). $C_{27}H_{32}FN_3O_7S$ requires 562.2023

4-Fluoro-N-((E)-(7S,10S,13S)-7-formyl-10-isopropyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraen-13-yl)benzenesulfonamide (5.36)

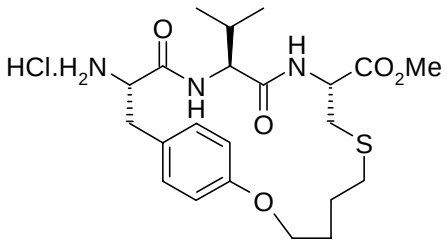


Methyl ester **5.35** (0.0469g, 0.0835 mmol) was reduced using General Procedure R. The crude material was purified by flash chromatography on silica using EtOAc to yield a brown solid, 0.00800g, 18%. R_f = 0.66 (EtOAc)

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 9.36 (1H, s, C**H**O), 8.12 (1H, d J=8.6Hz, N**H**), 7.90-8.00 (2H, m, Ar-**H** (4-F-Ph)),7.56 (1H, d J=8.9Hz, N**H**), 7.50 (1H, d J=7.4Hz, N**H**), 7.34-7.42 (1H, m, Ar-**H** (4-F-Ph)), 6.90 (2H, d J=8.4Hz, Ar-**H** (Tyr)), 6.62 (2H, d J=8.4Hz, Ar-**H** (Tyr)), 5.53-5.57 (1H, m, $OCH_2C\mathbf{H}CHCH_2$), 5.42-5.48 (1H, m, $OCH_2CHC\mathbf{H}CH_2$), 4.53-4.67 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.23-4.41 (2H, m, $C\mathbf{H}CO_2CH_3$ and $C\mathbf{H}CH_2Ph$), 3.69-3.82 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 2.63-2.84 (2H, m, $CHC\mathbf{H_2}Ph$), 2.26-2.31 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 2.15-2.19 (1H, m, $OCH_2CHCHC\mathbf{H_2}$), 1.52-1.61 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 0.64 (3H, d J=7.1Hz, $CHCH(C\mathbf{H_3})_2$), 0.57 (3H, d J=7.1Hz, $CHCH(C\mathbf{H_3})_2$)

HRMS (ES) 564.2172 (MH^+ (hemi-acetal). $C_{27}H_{34}FN_3O_7S$ requires 564.2180

(9R,12S,15S)-15-Amino-12-isopropyl-11,14-dioxo-2-oxa-7-thia-10,13-diaza-bicyclo-[15.2.2]-henicosa-1(20),17(21),18-triene-9-carboxylic acid methyl ester hydrochloride (5.42)



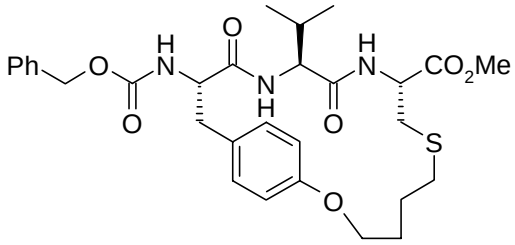
N-BOC protected compound **4.67** (0.300g, 0.544 mmol) was reacted using General Procedure I1 to afford an off white solid, 0.265g, 100%

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 8.66 (2H, bs, $N\mathbf{H_2}$), 8.54 (1H, d J=7.3Hz, N**H**), 8.18 (1H, d J=8.9Hz, N**H**), 6.98 (2H, d J=8.3Hz, Ar-**H**), 6.78 (2H, d J=8.3Hz, Ar-**H**), 4.38-4.42 (1H, m, $C\mathbf{H}CO_2CH_3$), 4.02-4.28 (4H, m, $C\mathbf{H}CH_2Ph$ and $OC\mathbf{H_2}CH_2CH_2CH_2S$ and $OCH_2CH_2CH_2C\mathbf{H_2}S$), 3.89-3.95 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.84-3.88 (1H, m, $OCH_2CH_2CH_2C\mathbf{H_2}S$), 3.55 (3H, s, $CHCO_2C\mathbf{H_3}$), 3.06-3.14 (2H, m, $CHC\mathbf{H_2}Ph$), 2.71-2.90 (2H, m,

CHC**H**$_2$S), 1.93-1.98 (1H, m, CHC**H**$(CH_3)_2$), 1.60-1.75 (4H, m, $OCH_2CH_2C\mathbf{H}_2CH_2S$ and $OCH_2C\mathbf{H}_2CH_2CH_2S$), 0.88 (3H, d J=7.1Hz, $CHCH(C\mathbf{H}_3)_2$), 0.87 (3H, d J=7.1Hz, $CHCH(C\mathbf{H}_3)_2$)

LRMS (ES) 452.3 (MH^+). $C_{22}H_{33}N_3O_5$ requires 452.2

(9R,12S,15S)-15-Benzyloxycarbonylamino-12-isopropyl-11,14-dioxo-2-oxa-7-thia-10,13-diazabicyclo-[15.2.2]henicosa-1(20),17(21),18-triene-9-carboxylic acid methyl ester (5.45)

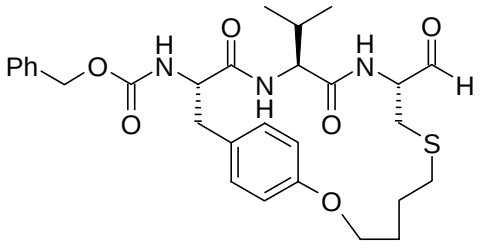


Amine **5.42** (0.200g, 0.410 mmol) was reacted with benzyl chloroformate using General Procedure H1. The crude material was purified by flash chromatography on silica using a gradient of EtOAc/DCM to yield an off-white solid, 0.0430g, 18%. R_f = 0.44 (30% EtOAc/DCM).

^{1}H NMR ppm (500 MHz in (CD_3OD)) (Compound exists as a mixture of rotamers) 7.17-7.50 (5H, m, Ar-**H** (CBZ)), 6.94-7.18 (2H, m, Ar-**H** (Tyr)), 6.62-6.84 (2H, m, Ar-**H** (Tyr)), 5.02 (1H, d J=10.6Hz, $OC\mathbf{H}_2Ph$), 4.98 (1H, d J=10.6Hz, $OC\mathbf{H}_2Ph$), 4.32-4.49 (1H, m, $C\mathbf{H}CO_2CH_3$), 4.14-4.33 (5H, m, $C\mathbf{H}CH_2Ph$ and $OC\mathbf{H}_2CH_2CH_2CH_2S$ and $OCH_2CH_2CH_2C\mathbf{H}_2S$), 3.80-3.94 (1H, m, $C\mathbf{H}CH(CH_3)_2$), 3.70 (3H, s, $CHCO_2C\mathbf{H}_3$), 2.88-3.12 (2H, m, $CHC\mathbf{H}_2Ph$), 2.65-2.90 (2H, m, $CHC\mathbf{H}_2S$), 2.02-2.10 (1H, m, $CHC\mathbf{H}(CH_3)_2$), 1.55-1.65 (4H, m, $OCH_2CH_2C\mathbf{H}_2CH_2S$ and $OCH_2C\mathbf{H}_2CH_2CH_2S$), 0.86-0.91 (6H, m, $CHCH(C\mathbf{H}_3)_2$),

HRMS (ES) 586.2566 (MH^+). $C_{30}H_{39}N_3O_7S$ requires 586.2587

(9R,12S,15S)-9-Formyl-12-isopropyl-11,14-dioxo-2-oxa-7-thia-10,13-diaza-bicyclo-[15.2.2]henicosa-1(20),17(21),18-trien-15-yl)-carbamic acid benzyl ester (5.46)



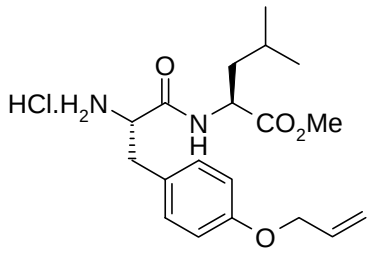
Methyl ester **5.45** (0.0430g, 0.0734 mmol) was reduced using General Procedure R. The crude material was purified by flash chromatography on silica using EtOAc to yield a brown solid, 0.00660g, 17%. R_f = 0.51 (EtOAc)

^{1}H NMR ppm (500 MHz in $(CD_3)_2SO$) 9.39 (1H, s, C**H**O), 7.70 (1 H, d J=9.1Hz, N**H** Cys), 7.66 (1H, d J=8.1Hz, N**H** Val), 7.12-7.43 (5H, m, Ar-**H** (CBZ)), 7.05 (2H, d J=7.8Hz, Ar-**H** (Tyr)), 6.78 (2H, d J=7.8Hz, Ar-**H** (Tyr)), 6.62 (1H, d J=8.3Hz, N**H**), 5.03 (2H, s, $OC\mathbf{H}_2Ph$), 4.20-4.32 (3H, m, $C\mathbf{H}CO_2CH_3$ and

OC**H$_2$**CH$_2$CH$_2$CH$_2$S), 4.05-4.15 (3H, m, C**H**CH$_2$Ph and OCH$_2$CH$_2$CH$_2$C**H$_2$**S), 3.85 (1H, dd J=6.5Hz, J=8.1Hz, C**H**CH(CH$_3$)$_2$), 2.78-2.90 (2H, m, CHC**H$_2$**Ph), 2.54-2.70 (2H, m, CHC**H$_2$**S), 1.94-2.00 (1H, m, CHC**H**(CH$_3$)$_2$), 1.21-1.71 (4H, m, OCH$_2$CH$_2$C**H$_2$**CH$_2$S and OCH$_2$C**H$_2$**CH$_2$CH$_2$S), 0.82-0.88 (6H, m, CHCH(C**H$_3$**)$_2$),

HRMS (ES) 586.2480 (MH^+). $C_{29}H_{37}N_3O_6S$ requires 556.2481

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-aminopropionylamino]-4-methylpentanoic acid methyl ester hydrogen chloride salt (6.1)



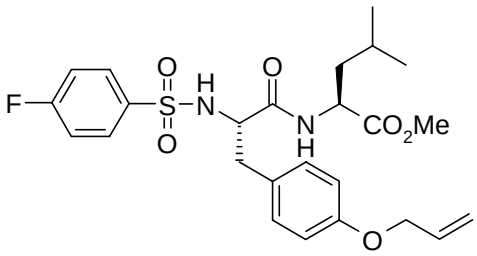
N-BOC protected compound **4.45** (4.00g, 8.92mmol) was reacted using General Procedure I1 to afford a white solid, 3.43g, 100%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.28 (2H, bs,N**H$_2$**), 7.67 (1H, d, J=6.4Hz N**H** Leu), 7.27 (2H, d J=8.4Hz, Ar-**H**), 6.82 (2H, d J=8.4Hz, Ar-**H**), 6.00 (1H, tdd J=5.2Hz, J=5.2Hz, J=10.3Hz, J=16.9Hz, CH_2C**H**CH_2), 5.22-5.40 (2H, m, CH_2CHC**H$_2$**), 4.57-4.64 (1H, m, C**H**CH_2Ph), 4.44 (2H, d J=5.2Hz, OC**H$_2$**$CHCH_2$), 4.28-4.37 (1H, m, C**H**$CH_2CH(CH_3)_2$), 3.63 (3H, s, CO_2C**H$_3$**), 3.36 (1H, dd J=4.3Hz, J=13.9Hz, CHC**H$_2$**Ph), 3.20 (1H, dd J=8.2Hz, J=13.9Hz, CHC**H$_2$**Ph), 1.53-1.63 (3H, m, CHC**H$_2$**$CH(CH_3)_2$ and $CHCH_2C$**H**$(CH_3)_2$), 0.83 (3H, d J=6.7 Hz, $CHCH_2CH(C$**H$_3$**$)_2$), 0.82 (3H, d J=6.7Hz, $CHCH_2CH(C$**H$_3$**$)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.4, 168.2, 157.9, 133.1, 131.0, 126.2, 117.5, 114.9, 68.6, 67.0, 54.6, 52.2, 51.4, 40.1, 36.2, 24.5, 22.5, 21.9.

LRMS (ES) 349.2 (MH^+). $C_{19}H_{28}N_2O_6$ requires 349.2

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-(4-fluorobenzenesulfonylamino)-propionylamino]-4-methylpentanoic acid methyl ester (6.2)



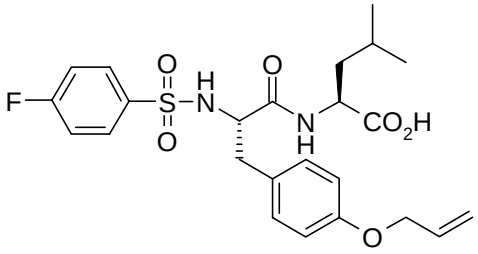
Amine **6.1** (3.50g, 9.10 mmol) was reacted with 4-fluoro-benzene sulfonyl chloride using General Procedure M2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 2.70g, 65%. R_f = 0.81 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.68-7.71 (2H, m, Ar-**H** (4-F-Ph)), 7.06-7.11 (2H, m, Ar-**H** (4-F-Ph)), 6.91 (2H, d J=8.6Hz, Ar-**H** (Tyr)), 6.73 (2H, d J=8.6Hz, Ar-**H** (Tyr)), 6.42 (1H, d J=8.2Hz, N**H** Leu), 6.05 (1H, tdd J=5.3Hz, J=5.3Hz, J=10.5Hz, J=17.2Hz, CH_2C**H**CH_2) 5.27-5.44 (2H, m, CH_2CHC**H$_2$**), 4.46-4.51 (3H, m, OC**H$_2$**$CHCH_2$, and C**H**$CH_2CH(CH_3)_2$), 3.84-3.88 (1H, m, C**H**CH_2Ph), 3.71 (3H, s, CO_2C**H$_3$**), 2.94 (1H, dd J=7.2Hz, J=14.4Hz, CHC**H$_2$**Ph), 2.90 (1H, dd J=6.4Hz, J=14.4Hz, CHC**H$_2$**Ph) 1.52-1.58 (1H, m, $CHCH_2C$**H**$(CH_3)_2$) 1.38-1.47 (2H, m, CHC**H$_2$**$CH(CH_3)_2$) 0.88 (6H, d, J=6.2Hz, CHC**H$_2$**$CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.5, 169.6, 157.9, 133.0, 130.2, 129.9, 129.8, 127.0, 117.8, 116.4, 116.1, 115.0, 68.7, 57.8, 52.3, 50.9, 41.4, 37.8, 24.6, 22.6, 21.8.

LRMS (ES) 507.3 (MH^+). $C_{25}H_{31}FN_2O_6S$ requires 507.2.

(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-(4-fluorobenzenesulfonylamino)propionylamino]-4-methylpentanoic acid (6.3)



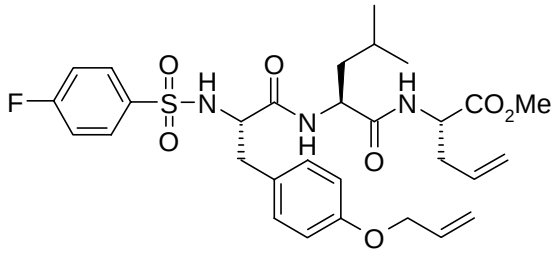
Dipeptide **6.2** (2.70g, 5.33 mmol) was dissolved in THF (30 mL) and hydrolysed using General Procedure C1 to afford a white solid, 2.50g, 95%.

^{1}H NMR ppm (500 MHz in CD_3OD) 8.27 (1H, d, J=8.0Hz, N**H** Leu), 7.60-7.63 (2H, m, Ar-**H** (4-F-Ph)), 7.04-7.08 (2H, m, Ar-**H** (4-F-Ph)) 7.02 (2H, d J=8.5Hz, Ar-**H** Tyr) 6.71 (2H, d J=8.5Hz Ar-**H** Tyr) 6.04 (1H, tdd J=5.2Hz, J=5.2Hz, J=10.5Hz, J=17.3Hz, OCH_2CHCH_2) 5.20-5.40 (2H, m, OCH_2CHCH_2), 4.49 (2H, d, J=5.2Hz, OCH_2CHCH_2), 4.22-4.27 (1H, m, $CHCH_2CH(CH_3)_2$), 3.99 (1H, dd, J=4.5Hz, J=9.5Hz, $CHCH_2Ph$), 2.96 (1H, dd, J=4.5Hz, J=14.0Hz, $CHCH_2Ph$), 2.65 (1H, dd, J=9.5Hz, J=14.0Hz, $CHCH_2Ph$), 1.45-1.56 (3H, m, $CHCH_2CH(CH_3)_2$and $CHCH_2CH(CH_3)_2$), 0.90 (3H, d J=5.8 Hz, $CHCH_2CH(CH_3)_2$), 0.84 (3H, d J=5.8 Hz, $CHCH_2CH(CH_3)_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 174.2, 172.0, 166.4, 157.6, 137.0, 133.7, 130.8, 130.1, 129.6, 129.4, 128.7, 116.1, 115.7, 115.4, 114.1, 68.4, 58.0, 50.6, 40.4, 37.9, 24.5, 22.0, 20.6.

LRMS (ES) 493.2 (MH^+). $C_{24}H_{29}FN_2O_6S$ requires 493.2

(S)-2-{(S)-2-[(S)-3-(4-Allyloxyphenyl)-2-(4-fluorobenzenesulfonylamino)propionylamino]-4-methyl-pentanoylamino}pent-4-enoic acid methyl ester (6.4)



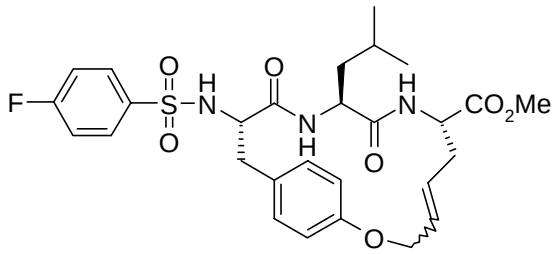
Carboxylic acid **6.3** (2.50g, 5.08 mmol) was reacted with amine **4.14** using General Procedure A2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 2.70g, 89%. R_f = 0.38 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in CD_3OD) 7.61-7.65 (2H, m, Ar-**H** (4-F-Ph)), 7.06-7.10 (2H, m, Ar-**H** (4-F-Ph), 6.97 (2H, d J=8.5Hz, Ar-**H** Tyr), 6.69 (2H, d J=8.5Hz, Ar-**H** Tyr), 6.05 (1H, tdd J=5.2Hz, J=5.2Hz, J=10.5Hz, J=17.3Hz, $OCH_2C\mathbf{H}CH_2$), 5.78 (1H, tdd J=7.1Hz, J=10.2Hz, J=17.1Hz, $CHCH_2C\mathbf{H}CH_2$), 5.04-5.42 (4H, m, $OCH_2CHC\mathbf{H_2}$ and $CHCH_2CHC\mathbf{H_2}$), 4.48 (2H, d J=5.2Hz, $OC\mathbf{H_2}CHCH_2$), 4.42 (1H, dd J=5.6Hz, J=8.0Hz, $C\mathbf{H}CO_2CH_3$), 4.32 (1H, dd J=5.0Hz, J=9.4Hz, $C\mathbf{H}CH_2CH(CH_3)_2$), 3.95 (1H, dd J=4.7Hz, J=9.5Hz, $C\mathbf{H}CH_2Ph$), 3.69 (3H, s, $CO_2C\mathbf{H_3}$), 2.93 (1H, dd J=4.7Hz, J=14.0Hz, $CHC\mathbf{H_2}Ph$), 2.63 (1H, dd J=9.5Hz, J=14.0Hz, $CHC\mathbf{H_2}Ph$), 2.41-2.57 (2H, m, $CHC\mathbf{H_2}CHCH_2$), 1.50-1.57 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 1.44-1.48 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 0.92 (3H, d J=6.0Hz, $CHCH_2CH(\mathbf{CH_3})_2$), 0.87 (3H, d J=6.0Hz, $CHCH_2CH(\mathbf{CH_3})_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 171.7, 171.5, 170.4, 167.3, 163.7, 157.7, 134.4, 134.3, 133.0, 132.3, 130.2, 129.9, 129.8, 127.2, 118.9, 117.7, 116.3, 116.0, 114.8, 68.6, 58.4, 52.2, 51.9, 51.9, 41.0, 37.5, 36.1, 24.6, 22.8, 21.9

LRMS (ES) 604.3 (MH^+). $C_{30}H_{38}FN_3O_7S$ requires 604.2

(*E/Z*)-(7S,10S,13S)-13-(4-Fluorobenzenesulfonylamino)-10-isobutyl-9,12-dioxo-2-oxa-8,11-diazabicyclo-[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (6.5)



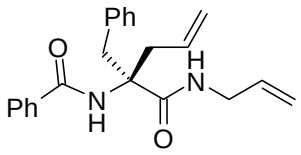
Diene **6.4** (1.18g, 1.95 mmol) was reacted with Grubbs second generation catalyst using General Procedure E2. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.700g, 63%. A 1:6.8 ratio of geometric isomers was obtained. R_f = 0.17 and 0.18 (1/1 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm for major isomer from mixture (500 MHz in $(CD_3)_2SO$) 8.18 (1H, d J=7.6Hz, N**H** Tyr), 8.08 (1H, d J=8.3Hz, N**H** Gly), 7.88-7.93 (2H, m, Ar-**H** (4-F-Ph)), 7.58 (1H, d J=7.5Hz, N**H** Leu), 7.32-7.38 (2H, m, Ar-**H** (4-F-Ph)), 6.90 (2H, d J=7.7Hz, Ar-**H** (Tyr)), 6.64 (2H, d J=7.7Hz, Ar-**H** (Tyr)), 5.52-5.56 (1H, m, $OCH_2C\mathbf{H}CHCH_2$), 5.39-5.46 (1H, m, $OCH_2CHC\mathbf{H}CH_2$), 4.53-4.67 (2H, m, $OC\mathbf{H_2}CHCHCH_2$), 4.20-4.30 (2H, m, $C\mathbf{H}CH_2Ph$ and $C\mathbf{H}CO_2CH_3$), 3.78-3.85 (1H, m, $C\mathbf{H}CH_2CH(CH_3)_2$), 3.54 (3H, s, $CO_2C\mathbf{H_3}$), 2.58-2.72 (2H, m, $CHC\mathbf{H_2}Ph$), 2.14-2.25 (2H, m, $OCH_2CHCHC\mathbf{H_2}$), 1.15-1.25 (2H, m, $CHC\mathbf{H_2}CH(CH_3)_2$), 0.99-1.05 (1H, m, $CHCH_2C\mathbf{H}(CH_3)_2$), 0.75 (3H, d J=6.8Hz, $CHCH_2CH(C\mathbf{H_3})_2$), 0.70 (3H, d J=6.8Hz, $CHCH_2CH(C\mathbf{H_3})_2$)

Selected ^{1}H NMR ppm for minor isomer from mixture: 7.15-7.22 (2H, m, Ar-**H** (4-F-Ph)), 7.02-7.06 (2H, m, Ar-**H** (Tyr)), 0.73 (3H, d J=6.8Hz, $CHCH_2CH(C\mathbf{H_3})_2$), 0.69 (3H, d J=6.8Hz, $CHCH_2CH(C\mathbf{H_3})_2$)

HRMS (ES) 576.2191 (MH^+). $C_{28}H_{34}FN_3O_7S$ requires 576.2180

N-((R)-1-Allylcarbamoyl-1-benzylbut-3-enyl)-benzamide (7.6)

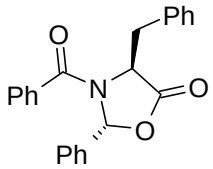


Allyl amine (0.283 mL, 3.78 mmol) was dissolved in anhydrous THF (10 mL) under an atmosphere of argon. The mixture was cooled to -78^{o}C, ^{n}BuLi (1 equiv) was added and stirred at -78^{o}C for five min. A solution of oxazolidinone **7.12** (0.33 equiv) in anhydrous THF (10 mL) was added and stirring continued at -78^{o}C for one h and then at rt for a further seventeen h. The reaction was quenched with saturated $NH_4Cl_{(aq)}$ (2 mL) and concentrated *in vacuo*. The residue was partitioned between EtOAc and saturated $NaHCO_{3(aq)}$. The organic phase was washed with saturated $NH_4Cl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a white solid, 0.296g, 91%. R_f = 0.17 (1/9 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.67 (2H, dd J=3.3Hz, J=5.2Hz, Ar-**H**), 7.46-7.50 (1H, m, Ar-**H**), 7.37-7.42 (2H, m, Ar-**H**), 7.19-7.21 (3H, m, Ar-**H**), 7.13 (2H, dd J=2.8Hz, J=6.7Hz, Ar-**H**), 6.92 (1H, dd J=6.2Hz, J=11.2Hz, N**H**CH_2CHCH_2), 5.83 (1H, tdd J=5.8Hz, J=5.8Hz, J=10.3Hz, J=17.1Hz, $NHCH_2C$**H**CH_2), 5.68-5.77 (1H, m, CH_2C**H**CH_2), 5.11-5.23 (4H, m, $NHCH_2CHC$**H$_2$** and $NHCH_2CHC$**H$_2$**), 3.96 (1H, ddd J=5.8Hz, J=5.8Hz, J=15.4Hz, NHC**H$_2$**$CHCH_2$), 3.89 (1H, ddd J=5.7Hz, J=5.7Hz, J=15.5Hz, NHC**H$_2$**$CHCH_2$), 3.75 (1H, d J=13.9Hz, C**H$_2$**Ph), 3.37 (1H, d J=13.8Hz, C**H$_2$**Ph), 3.34 (1H, dd J=7.9Hz, J=14.4Hz, C**H$_2$**$CHCH_2$), 2.79 (1H, dd J=6.5Hz, J=14.3Hz, C**H$_2$**$CHCH_2$)

LRMS (ES) 349.2 (MH^+). $C_{22}H_{24}N_2O_2$ requires 349.2

(2R,4S)-3-Benzoyl-4-benzyl-2-phenyloxazolidin-5-one (7.9) Lit[1]



(L)-phenylalanine (10.0g, 60.5 mmol) was suspended in 1M $NaOH_{(aq)}$ (1 equiv). This was stirred at rt for ten min and then the resultant colourless solution was concentrated *in vacuo*. The residue was suspended in DCM (400 mL), and benzaldehyde (1.5 equiv) added. This was refluxed for eighteen h during which time water was removed azetropically using Dean-Stark apparatus. The reaction mixture was cooled in ice and benzoyl chloride (1 equiv) was added. This was stirred in ice for four h and then at rt for a further twelve h. This was partitioned with 5% $NaHCO_{3(aq)}$ and the organic phase was washed sequentially with 5% $KHSO_{4(aq)}$, water and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was recrystallised from hot methanol to afford a white solid, 12.8g, 59%

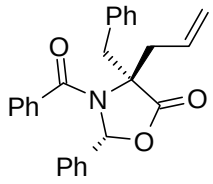
mp 181-183^{o}c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.04-7.38 (15H, m, Ar-**H**), 5.80 (1H, s, C**H**Ph), 5.21 (1H, bs, C**H**CH_2Ph), 3.77 (1H, bs, CHC**H$_2$**Ph), 3.41 (1H, bs, CHC**H$_2$**Ph)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 171.0, 169.2, 136.2, 135.2, 130.8, 129.8, 128.7, 128.5, 127.7, 126.7, 91.2, 57.6, 34.9

LRMS (ES) 358.1 (MH^+). $C_{23}H_{19}NO_3$ requires 358.1

(2R,4R)-4-Allyl-3-benzoyl-4-benzyl-2-phenyloxazolidin-5-one (7.10) Lit[1]



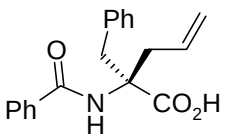
Oxazolidinone **7.9** (1.00g, 2.80 mmol) was dissolved in anhydrous THF (20 mL) under an atmosphere of argon. The mixture was cooled to -78^{o}C, a solution of 1M lithium bis(trimethylsilyl)amide in THF (1.1 equiv) added and stirring continued at -78^{o}C for three min before allyl bromide (1.5 equiv) was added. The mixture was stirred at -78^{o}C for a further two h and then at rt for a further sixteen h before being concentrated *in vacuo*. The residue was partitioned between EtOAc and saturated $NaHCO_{3(aq)}$. The organic phase was washed with saturated $NH_4Cl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a yellow solid, 1.03g, 93%
mp 140-142^{o}c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.37-7.46 (5H, m, Ar-**H**), 7.14 (1H, dd J=0.9Hz, J=7.5Hz, Ar-**H**), 7.00-7.08 (2H, m, Ar-**H**), 6.92-6.99 (1H, m, Ar-**H**), 6.78 (2H, dd J=0.9Hz, J=7.2Hz, Ar-**H**), 6.65-6.72 (2H, m, Ar-**H**), 6.04 (1H, s, C**H**Ph), 5.91-6.02 (1H, m, CH_2C**H**CH_2), 5.46-5.59 (4H, m, CH_2C**H**CH_2 and Ar-**H**), 4.10 (1H, d J=13.5 Hz, C**H$_2$**Ph), 3.61 (1H, dd J=9.8Hz, J=13.8Hz, C**H$_2$**$CHCH_2$), 3.46 (1H, d J=13.4 Hz, C**H$_2$**Ph), 2.83 (1H, dd J=5.2Hz, J=13.7Hz, C**H$_2$**$CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 173.1, 169.8, 136.6, 136.3, 135.0, 131.2, 130.8, 129.1, 129.0, 128.2, 127.7, 127.6, 127.4, 125.2, 122.1, 91.0, 70.0, 41.6, 39.7

LRMS (ES) 398.2 (MH^+). $C_{26}H_{23}NO_3$ requires 398.2

(R)-2-Benzoylamino-2-benzyl-pent-4-enoic acid (7.11)



Oxazolidinone **7.10** (0.900g, 2.26 mmol) was dissolved in a 4:1 methanol/water mixture (25 mL) a and sodium hydroxide (2 equiv) was added. The mixture was refluxed for two h and then concentrated *in vacuo*. The residue was partitioned between EtOAc and 1M $HCl_{(aq)}$. The aqueous phase was extracted twice more with

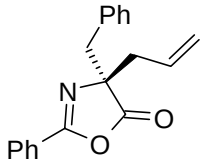
EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated *in-vacuo* to afford a white solid, 0.496g, 100%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.68 (2H, dd J=3.0Hz, J=10.6Hz, Ar-**H**), 7.40-7.53 (3H, m, Ar-**H**), 7.18-7.27 (5H, m, Ar-**H**), 5.66 (1H, tdd J=7.4Hz, J=7.4Hz, J=10.0Hz, J=14.6Hz, $CH_2C\mathbf{H}CH_2$), 5.04-5.21 (2H, m, $CH_2CHC\mathbf{H_2}$), 3.86 (1H, d J=12.1Hz, $C\mathbf{H_2}Ph$), 3.43 (1H, dd J=7.6Hz, J=13.1Hz, $C\mathbf{H_2}CHCH_2$), 3.35 (1H, d J=13.0Hz, $C\mathbf{H_2}Ph$), 2.81 (1H, dd J=8.3Hz, J=13.5Hz, $C\mathbf{H_2}CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 176.8 , 154.4, 136.5, 135.7 , 131.7 , 129.8, 128.4, 128.2, 128.1, 126.9, 119.5, 66.4, 64.8, 40.4, 39.8

LRMS (ES) 310.2 (MH^+). $C_{19}H_{19}NO_3$ requires 310.1

(R)-4-Allyl-4-benzyl-2-phenyl-4H-oxazol-5-one (7.12)

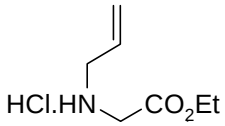


Carboxylic acid **7.11** (0.115g, 0.372 mmol) was dissolved in anhydrous DCM under an atmosphere of argon. The mixture was cooled to -20^{o}C, anhydrous pyridine (1 equiv) and cyanuric fluoride (5 equiv) were added. The mixture was stirred at -20^{o}C for one h and at rt for a further seventeen h. The resultant white precipitate was removed by suction filtration. The residue was washed with DCM and the filtrate was partitioned with water. The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a yellow oil, 0.108g, 100%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.84 (2H, dd J=1.4Hz, J=7.6Hz, Ar-**H**), 7.50-7.55 (1H, m, Ar-**H**), 7.44-7.49 (2H, m, Ar-**H**), 7.17-7.19 (5H, m, Ar-**H**), 5.69-5.79 (1H, m, $CH_2C\mathbf{H}CH_2$), 5.15-5.30 (2H, m, $CH_2CHC\mathbf{H_2}$), 3.31 (1H, d J=13.5Hz, $C\mathbf{H_2}Ph$), 3.23 (1H, d J=13.5Hz, $C\mathbf{H_2}Ph$), 2.78 (1H, dd J=6.9Hz, J=13.7Hz, $C\mathbf{H_2}CHCH_2$), 2.72 (1H, dd J=8.0Hz, J=13.5Hz, $C\mathbf{H_2}CHCH_2$)

LRMS (ES) 292.1332 (MH^+). $C_{19}H_{17}NO_2$ requires 292.1337

Allylamino-acetic acid ethyl ester hydrochloride (7.13) Lit[2]

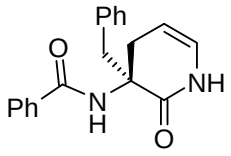


Allyl amine (3.72 mL, 49.6 mmol) was dissolved in anhydrous THF (105 mL). The mixture was cooled in ice and a solution of ethyl bromoacetate (0.5 equiv) in THF (40 mL) was added dropwise over forty five min. Stirred was continued in ice for one h and then at rt for a further ninety min. The reaction mixture was concentrated *in vacuo* and the residue suspended in diethyl ether (75 mL). This was stirred at rt for ten min and then filtered under vacuum. The filtrate was concentrated *in vacuo* to afford a yellow oil, 2.59g, 73%.

^{1}H NMR ppm (300 MHz in $CDCl_3$) 5.85 (tdd J=6.1HZ, J=6.1Hz, J=10.2, J=17.2Hz, $CH_2C\mathbf{H}CH_2$), 5.07-5.22 (2H, m, $CH_2CHC\mathbf{H_2}$), 4.18 (2H, q J=7.2Hz, $CO_2C\mathbf{H_2}CH_3$), 3.38 (2H, s, $C\mathbf{H_2}CO_2Et$), 3.26 (1H, dd J=1.4Hz, J=1.4Hz, $C\mathbf{H_2}CHCH_2$), 3.24 (1H, dd J=1.3Hz, J=1.3Hz, $C\mathbf{H_2}CHCH_2$), 1.26 (3H, t J=7.2Hz, $CO_2CH_2C\mathbf{H_3}$)

LRMS (ES) 144.1 (MH^+). $C_7H_{13}NO_2$ requires 144.1

N-((R)-3-Benzyl-2-oxo-1,2,3,4-tetrahydro-pyridin-3-yl)benzamide (7.14)

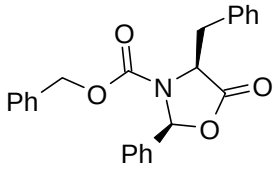


Diene **7.6** (0.0480g, 0.138 mmol) was dissolved in benzene (10 mL), TFA (1 mL) and Grubbs second generation catalyst (0.1 equiv) were added. The mixture was refluxed for eighteen h and then concentrated *in-vacuo*. The crude material was purified by flash chromatography on silica using a gradient of methanol and DCM to yield a brown solid, 0.0141g, 32%. R_f = 0.09 (2% MeOH/DCM).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.63 (2H, d J=7.1Hz, Ar-**H**), 7.32-7.45 (3H, m, Ar-**H**), 7.15-7.18 (3H, m, Ar-**H**), 7.09-7.11 (2H, m, Ar-**H**), 5.62-5.73 (2H, m, NHC**H**$CHCH_2$ and $NHCHC\mathbf{H}CH_2$), 5.11 (1H, d J=8.0Hz, N**H**$CHCHCH_2$), 4.97 (1H, d J=8.9Hz, N**H**C(O)Ph), 3.83 (1H, dd J=10.5Hz, J=16.2Hz, $NHCHCHC\mathbf{H_2}$), 3.42-3.50 (1H, m, $NHCHCHC\mathbf{H_2}$), 3.28 (1H, d, J=12.5Hz, $C\mathbf{H_2}$Ph), 2.76 (1H, d J=12.5Hz, $C\mathbf{H_2}$Ph)

LRMS (ES) 307.1 (MH^+). $C_{19}H_{18}N_2O_2$ requires 307.2

(2S,4S)-4-Benzyl-5-oxo-2-phenyloxazolidine-3-carboxylic acid benzyl ester (7.16) Lit[3]

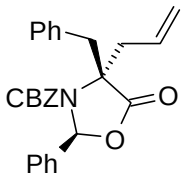


(L)-N-CBZ-Phe (4.00g, 13.4 mmol) was dissolved in anhydrous diethyl ether (70 mL) under an atmosphere of argon. The mixture was cooled to -78^{o}C and benzaldehyde dimethylacetyl (0.96 equiv) and boron trifluoride etherate (4.9 equiv) were added. Stirring was continued at -78^{o}C for one h and then at rt for a further seventeen h. The reaction was quenched with saturated $NaHCO_{3(aq)}$ (50 mL) and the mixture was allowed to partition. The aqueous phase was extracted twice more with diethyl ether and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of diethyl ether, EtOAc and (50/70) pet ether to yield a white solid, 1.60g, 31%. R_f = 0.24 (5/1/20 (diethyl ether/EtOAc/(50/70) pet ether)).
mp 122-124^{o}c.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.05-7.45 (13H, m, Ar-**H**), 6.42-6.60 (2H, bm, Ar-**H**), 5.21 (1H, d J=12.2 Hz, $OC\mathbf{H_2}$Ph), 5.10 (1H, d J=12.2 Hz, $OC\mathbf{H_2}$Ph), 4.70 (1H, s, NC**H**O), 3.30-3.41 (2H, m, $C\mathbf{H_2}$Ph)

LRMS (ES) 388.2 (MH^+). $C_{24}H_{21}NO_4$ requires 388.2

(2S,4S)-4-Allyl-4-benzyl-5-oxo-2-phenyloxazolidine-3-carboxylic acid benzyl ester (7.17)

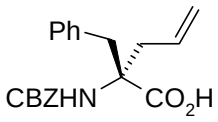


Oxazolidinone **7.16** (0.500g, 1.29 mmol) was dissolved in anhydrous THF (10 mL) under an atmosphere of argon. The mixture was cooled to -78^{o}C and a solution of 1M lithium bis(trimethylsilyl)amide in THF (1.1 equiv) was added. Stirring was continued at -78^{o}C for three min and then allyl bromide (1.5 equiv) was added. The mixture was stirred at -78^{o}C for two h and then at rt for a further sixteen h before being concentrated *in vacuo.* The residue was partitioned between EtOAc and saturated $NaHCO_{3(aq)}$. The organic phase was washed with saturated $NH_4Cl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a yellow solid, 0.275g, 50%. R_f = 0.26 (1/9 (EtOAc / (50/70) pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) was obtained which shows the presence of two rotamers about the carbamate bond in a ratio of 3:1 (Lit[4])
7.12-7.48 (12H, m, Ar-**H**), 6.95-7.04 (2H, m, Ar-**H**), 6.77 (1H, d J=7.1 Hz, Ar-**H**), 6.12 and 6.28 (1H, d J=7.1Hz, Ar-**H**), 6.05 (1H, s, NC**H**O), 5.69-5.88 (1H, m, $CH_2C\mathbf{H}CH_2$), 5.21-5.37 (2H, m, $CH_2CHC\mathbf{H_2}$), 5.08 (1H, d J=12.2Hz, O$C\mathbf{H_2}$Ph), 4.88 (1H, d J=12.1Hz, O$C\mathbf{H_2}$Ph), 3.66 (1H, d J=13.6Hz, $C\mathbf{H_2}$Ph), 3.29-3.39 (1H, m, $C\mathbf{H_2}$Ph and $C\mathbf{H_2}CHCH_2$), 2.68 (1H, dd J=6.1Hz, J=13.6Hz, $C\mathbf{H_2}CHCH_2$)

LRMS (ES) 428.2 (MH^+). $C_{27}H_{25}NO_4$ requires 428.2

(S)-2-Benzyl-2-benzyloxycarbonylaminopent-4-enoic acid methyl ester (7.18)



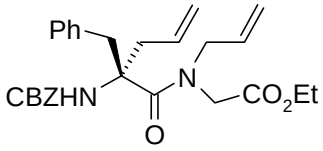
Oxazolidinone **7.17** (0.275g, 0.643 mmol) was dissolved in THF (20 mL) and hydrolysed using General Procedure C1. This afforded a white solid, 0.205g, 94%

^{1}H NMR ppm (500 MHz in $CDCl_3$) 8.95 (1H, bs, $CO_2\mathbf{H}$), 7.35-7.38 (5H, m, Ar-**H**), 7.14-7.20 (3H, m, Ar-**H**), 7.01 (2H, d J=7.0Hz, Ar-**H**), 5.66 (tdd, J=7.4Hz, J=7.4Hz, J=10.0Hz, J=15.1Hz, $CH_2C\mathbf{H}CH_2$), 5.56 (1H, bs, N**H**), 5.05-5.20 (4H, m, $CH_2CHC\mathbf{H_2}$ and O$C\mathbf{H_2}$Ph), 3.59 (1H, d J=13.6Hz, $C\mathbf{H_2}$Ph), 3.21 (1H, dd J=7.5Hz, J=13.9Hz, $C\mathbf{H_2}CHCH_2$), 3.17 (1H, d J=13.6Hz, $C\mathbf{H_2}$Ph), 2.65 (1H, dd J=7.2Hz, J=13.9Hz, $C\mathbf{H_2}CHCH_2$)

^{13}C NMR ppm (75 MHz in $CDCl_3$) 176.8, 154.4, 144.2, 136.5, 135.7, 131.7, 130.7, 129.8, 129.0, 128.4, 128.2, 128.1, 126.9, 119.5, 66.4, 64.8, 40.4, 39.8

LRMS (ES) 340.1 (MH^+). $C_{20}H_{21}NO_4$ requires 340.2

[Allyl-((S)-2-benzyl-2-benzyloxycarbonylaminopent-4-enoyl)-amino]-acetic acid ethyl ester (7.19)

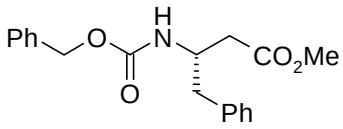


Carboxylic acid **7.18** (0.0500g, 0.147 mmol), amine **7.13** (1.7 equiv) and PyAOP (1.3 equiv) were dissolved in anhydrous DMF. DIPEA (2.7 equiv) was added and the reaction mixture stirred at rt for 18 h. This was partitioned between EtOAc and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield an orange oil, 0.0123g, 18%. R_f = 0.43 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.33-7.41 (5H, m, Ar-**H**), 7.26-7.31 (3H, m, Ar-**H**), 7.13-7.18 (2H, m, Ar-**H**), 6.04-6.13 (1H, m, $NCH_2C\mathbf{H}CH_2$), 5.63-5.78 (1H, m, $CH_2C\mathbf{H}CH_2$), 5.04-5.39 (6H, m, $NCH_2CHC\mathbf{H_2}$ and $CH_2CHC\mathbf{H_2}$ and $OC\mathbf{H_2}Ph$), 4.12 (2H, q J=7.1Hz, $CO_2C\mathbf{H_2}CH_3$), 3.70 (1H, d J=14.0Hz, $NC\mathbf{H_2}CHCH_2$), 3.60 (1H, d J=13.9Hz, $NC\mathbf{H_2}CHCH_2$), 3.11-3.15 (3H, m, $C\mathbf{H_2}CO_2Et$ and $C\mathbf{H_2}Ph$), 3.01 (1H, d J=14.0, $C\mathbf{H_2}Ph$), 2.80 (1H, dd J=5.5Hz, J=5.5Hz, $C\mathbf{H_2}CHCH_2$), 2.69 (1H, dd J=7.4Hz, J=14.0Hz, $C\mathbf{H_2}CHCH_2$), 2.58 (1H, dd J=7.2Hz, J=14.0Hz, $C\mathbf{H_2}CHCH_2$), 1.26 (3H, t J=7.1Hz, $CO_2CH_2C\mathbf{H_3}$)

HRMS (ES) 487.2201 (MH^+). $C_{27}H_{32}N_2O_5Na$ requires 487.2209

(S)-3-Benzyloxycarbonylamino-4-phenylbutyric acid methyl ester (7.25)



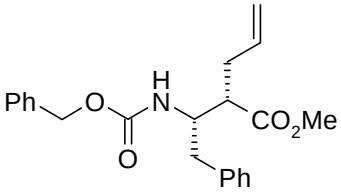
N-CBZ-Phe-H (5.00g, 16.7 mmol) was homologated using General Procedure P. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a colourless oil, 2.62g, 48%. R_f = 0.48 (1/3 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.08-7.34 (10H, m, Ar-**H**), 5.41 (1H, d J=8.6Hz, N**H**), 5.05 (2H, s, $OC\mathbf{H_2}Ph$), 4.15-4.28 (1H, m, $C\mathbf{H}CH_2Ph$), 3.63 (3H, s, $CO_2C\mathbf{H_3}$), 2.94 (1H, dd J=6.5Hz, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.81 (1H, dd J=7.6Hz, J=13.3Hz, $CHC\mathbf{H_2}Ph$), 2.49 (2H, t J=5.3Hz, $C\mathbf{H_2}CO_2CH_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 172.0, 155.7, 137.5, 136.5, 135.7, 133.2, 129.3, 128.5, 128.5, 128.1, 128.0, 126.7, 66.6, 51.7, 49.4, 40.2, 37.4

LRMS (ES) 328.1 (MH^+). $C_{19}H_{21}NO_4$ requires 328.2

(S)-2-((S)-1-tertButoxycarbonylamino-2-phenylethyl)pent-4-enoic acid methyl ester (7.26)



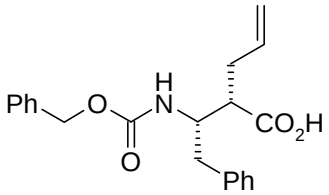
Methyl ester **7.25** (1.00g, 3.05 mmol) was allylated using General Procedure Q. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield an orange solid, 0.730g, 65%. R_f = 0.42 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.18-7.40 (10H, m, Ar-**H**), 5.84 (1H, d J=9.6Hz, N**H**), 5.62-5.72 (1H, m, $CHCH_2C\mathbf{H}CH_2$), 4.94-5.17 (2H, m, $CHCH_2CHC\mathbf{H_2}$), 4.06-4.17 (1H, m, $C\mathbf{H}CH_2Ph$), 3.71 (1H, s, $CO_2C\mathbf{H_3}$), 2.92 (1H, dd J=6.3Hz, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.70 (1H, dd J=8.5Hz, J=13.5Hz, $CHC\mathbf{H_2}Ph$), 2.60-2.66 (1H, m, $CHC\mathbf{H_2}CHCH_2$), 2.38-2.47 (1H, m, $CHC\mathbf{H_2}CHCH_2$), 2.30 (1H, td J=6.9HZ, J=6.9Hz, J=13.8Hz, $C\mathbf{H}CO_2CH_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 174.7, 155.9, 137.4, 136.6, 134.3, 129.2, 128.4, 127.9, 127.9, 126.5, 117.4, 66.5, 53.1, 51.6, 46.4, 40.5, 34.3

LRMS (ES) 368.2 (MH^+). $C_{22}H_{25}NO_4$ requires 368.2

(S)-2-((S)-1-tertButoxycarbonylamino-2-phenylethyl)pent-4-enoic acid (7.27)



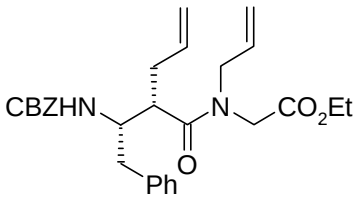
Methyl ester **7.26** (0.201g, 0.547 mmol) was hydrolysed using General Procedure C1 to afford an yellow solid, 0.160g, 83%.

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.05-7.32 (10H, m, Ar-**H**), 5.53-5.71 (1H, m, $CHCH_2C\mathbf{H}CH_2$), 4.81-5.16 (4H, m, $CHCH_2CHC\mathbf{H_2}$ and $CHC\mathbf{H_2}Ph$), 3.98-4.12 (1 H, m, $C\mathbf{H}CH_2Ph$), 2.88 (1H, dd J=6.5HZ, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.70 (1H, dd J=8.6Hz, J=13.7Hz, $CHC\mathbf{H_2}Ph$), 2.51-2.62 (1H, m, $CHC\mathbf{H_2}CHCH_2$), 2.35-2.41 (1H, m, $CHC\mathbf{H_2}CHCH_2$), 2.26 (1H, td, J= 7.2Hz, J=7.2Hz, J=14.6Hz, $C\mathbf{H}CO_2CH_3$)

^{13}C NMR ppm (75 MHz in $CDCl_3$). 178.9, 156.1, 137.4, 136.3, 134.1, 129.2, 128.4, 128.0, 126.5, 117.7, 66.7 , 52.9, 46.4, 40.5, 34.1

LRMS (ES) 354.2 (MH^+). $C_{21}H_{23}NO4$ requires 354.2

{Allyl-[(S)-2-((S)-1-benzyloxycarbonylamino-2-phenylethyl)pent-4-enoyl]amino}acetic acid ethyl ester (7.28)



Carboxylic acid **7.27** (0.0640, 0.181 mmol), amine **7.13** (1.7 equiv) and PyAOP (1.3 equiv) were dissolved in anhydrous DMF. DIPEA was added (2.7 equiv) and the reaction mixture stirred at rt for 18 h. This was partitioned between EtOAc and 1M $HCl_{(aq)}$. The organic phase was washed sequentially with 1M $HCl_{(aq)}$ and brine before being dried ($MgSO_4$), filtered and concentrated *in-vacuo*. The crude material was purified by flash chromatography on silica using a gradient of MeOH and DCM to yield a yellow oil, 0.0250g, 29%. R_f = 0.21 (2% MeOH / DCM).

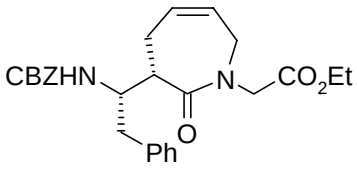
^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.12-7.38 (10H, m, Ar-**H**), 6.67 (1H, d J=9.5Hz, N**H**), 5.60-5.80 (2H, m, $CHCH_2C\mathbf{H}CH_2$ and $NCH_2C\mathbf{H}CH_2$), 4.96-5.24 (6H, m, $CHCH_2CHC\mathbf{H_2}$ and $NCH_2CHC\mathbf{H_2}$ and $OC\mathbf{H}_2Ph$), 4.22 (2H, q J=7.1Hz, $OC\mathbf{H}_2CH_3$), 4.14-4.19 (1H, m, $C\mathbf{H}CH_2Ph$), 4.13 (1H, d J=17.6Hz, $C\mathbf{H_2}CO_2CH_2CH_3$), 3.90 (1H, d J=17.1Hz, $C\mathbf{H_2}CO_2CH_2CH_3$), 3.79 (1H, d J=5.6Hz, $NC\mathbf{H_2}CHCH_2$), 2.97 (1H, dd, J=6.7, J=13.6Hz, $CHC\mathbf{H_2}Ph$), 2.76-2.82 (1H, m, $CHC\mathbf{H}CO$), 2.71 (1H, dd J=9.1Hz, J=13.6 Hz, $CHC\mathbf{H_2}Ph$), 2.28-2.42 (2H, m, $CHC\mathbf{H_2}CHCH_2$), 1.29 (3H, t J=7.1Hz, $OCH_2C\mathbf{H}_3$).

^{13}C NMR ppm (75 MHz in $CDCl_3$). 175.3, 169.0, 156.2, 138.2, 136.8, 134.5, 132.4, 129.1, 128.4, 128.3, 127.8, 127.7, 126.4, 118.5, 117.7, 66.2, 61.2, 53.3, 51.9, 47.0, 41.36, 40.0, 34.4, 14.1.

HRMS (ES) 479.2542 (MH^+). $C_{28}H_{35}N_2O_5$ requires 479.2546

FTIR CM^{-1} (KBr) 3402, 2945, 1747, 1717, 1635

[(S)-3-((S)-1-Benzyloxycarbonylamino-2-phenyl-ethyl)-2-oxo-2,3,4,7-tetrahydro-azepin-1-yl]acetic acid ethyl ester (7.29)



Diene **7.28** (0.0210g, 0.00438 mmol) was dissolved in anhydrous DCM (3 mL) under an atmosphere of argon and Grubbs second generation catalyst (0.1 equiv) was added. This was refluxed for eighteen h and then concentrated *in vacuo*. The crude material was purified by flash chromatography on silica using a gradient of EtOAc and (50/70) pet ether to yield a brown solid, 0.00430g, 22%. R_f = 0.31 (1/4 (EtOAc / (50/70) Pet ether)).

^{1}H NMR ppm (500 MHz in $CDCl_3$) 7.16-7.38 (10H, m, Ar-**H**), 6.62 (1H, d J=9.9Hz, N**H**), 5.68-5.74 (1H, m, $CHCH_2C\mathbf{H}CHCH_2N$), 5.58-5.64 (1H, m, $CHCH_2C\mathbf{H}CHCH_2N$), 5.10 (1H, d J=12.3Hz, $OC\mathbf{H}_2Ph$), 5.07 (1H, d J= 12.3Hz, $OC\mathbf{H}_2Ph$), 4.52 (1H, d J=17.1Hz, $C\mathbf{H_2}CO_2CH_2CH_3$), 4.24 (2H, q J=7.1Hz, $OC\mathbf{H}_2CH_3$), 4.18-4.28 (1H,

m, $CHCH_2CHCHC\mathbf{H_2}N$), 3.95 (1H, dddd J=2.8Hz, J=5.9Hz, J=8.7Hz, J=10.1Hz, $CHCH_2CHCHC\mathbf{H_2}N$), 3.86 (1H, d J= 17.1 Hz, $C\mathbf{H_2}CO_2CH_2CH_3$), 3.23 (1H, dd, J=5.9Hz, J=17.4Hz, $CHCH_2CHCHC\mathbf{H_2}N$), 3.09 (1H, dt J= 2.8, J=13.1Hz, $C\mathbf{H}CH_2CHCHCH_2N$), 3.03 (1H, dd J=5.5Hz, J=13.1Hz, $CHC\mathbf{H_2}Ph$), 2.92 (1H dd J=9.9Hz, J=13.1Hz, $CHC\mathbf{H_2}Ph$), 2.42-2.52 (1H, m, $CHC\mathbf{H_2}CHCHCH_2N$), 2.10-2.20 (1H, m, $CHC\mathbf{H_2}CHCHCH_2N$), 1.30 (3H, t J=7.1Hz, $OCH_2C\mathbf{H}_3$).

^{13}C NMR ppm (75 MHz in $CDCl_3$). 175.4, 169.1, 156.6, 138.7, 136.8, 131.2, 129.3, 128.6, 128.4, 127.8, 127.7, 126.4, 123.3, 66.4, 61.3, 55.9, 49.8, 47.3, 40.6, 39.9, 30.0, 14.2.

HRMS (ES) 451.2252 (MH^+). $C_{26}H_{31}N_2O_5$ requires 451.2233

FTIR CM^{-1} (KBr) 3410, 2981, 1744, 1717, 1647

References for Chapter 7 experimental

1. Gardiner, J.; Abell, A. D., Synthesis and solid state conformation of phenylalanine mimetics constrained in a proline-like conformation. *Organic & Biomolecular Chemistry* **2004,** 2, (16), 2365-2370.

2. Hong, B.-C.; Chen, Z.-Y.; Nagarajan, A.; Kottani, R.; Chavan, V.; Chen, W.-H.; Jiang, Y.-F.; Zhang, S.-C.; Liao, J.-H.; Sarshar, S., Efficient and stereo-divergent synthesis of deoxy-imino sugars. *Carbohydrate Research* **2005,** 340, (16), 2457-2468.

3. Cheng, H.; Keitz, P.; Jones, J. B., Design and Synthesis of a Conformationally Restricted Cysteine Protease Inhibitor. *Journal of Organic Chemistry* **1994,** 59, (25), 7671-6.

4. Khalil, E. M.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L., Synthesis and Dopamine Receptor Modulating Activity of Substituted Bicyclic Thiazolidine Lactam Peptidomimetics of L-Prolyl-L-leucyl-glycinamide. *Journal of Medicinal Chemistry* **1999,** 42, (15), 2977-2987.

Appendix 1: Calpain inhibition assay

The biological activity of calpain inhibitors synthesised in this thesis was determined by Dr Matthew Jones and Janna Nikkel using established protocols[1] to determine their inhibition constants (IC_{50}). IC_{50} indicates the concentration of inhibitor required to decrease the activity of the protease by 50%. This data was calculated using protease substrate casein labelled with fluorophore 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionic acid (BODIPY).

As shown schematically in **Figure A1,** without proteolysis no fluorescence is observed as auto-quenching of adjacent fluorophores occurs. When calpain is activated, proteolysis of the substrate occurs and fluorescence is detected.

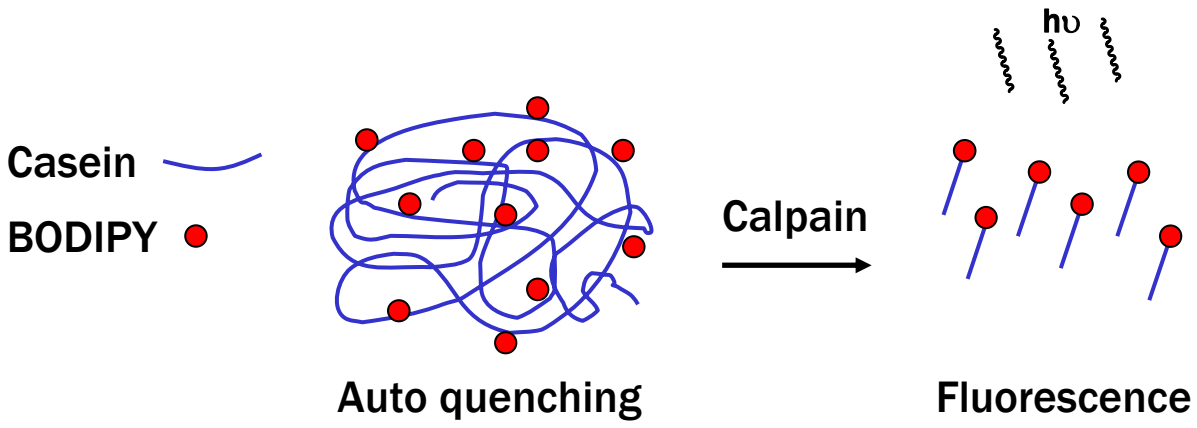


Figure A1: Schematic illustration of calpain IC_{50} assay

Calculating the IC_{50}

Assays were performed using calpain II, partially purified from sheep lung by ion-exchange chromatography, diluted to give a linear response over the course of the assay. The substrate

solution (0.0005% BODIPY-FL casein in 10 mM tris-HCl, pH 7.5 containing 10 mM $CaCl_2$, 0.1 mM NaN_3, and 0.1% mercaptoethanol) was prepared fresh each day.

The assays were performed in 96-well black ELISA plates. Calpain control assays contained 50 µL calpain II in sample buffer (20 mM tris-HCl, pH 7.5 containing 1 mM EDTA, 1 mM EGTA, and 2 mM dithiothreitol). Fifty µL of substrate solution was used to start the reaction. The progress of the reaction at 25°C was followed for 10 min in a BMG Fluorostar with excitation of 485 nm and emission of 530 nm. For the inhibitor assays the sample buffer was replaced by 50 µL of inhibitor diluted in the sample buffer. The percentage inhibition was determined as 100 times the activity with inhibitor present divided by the activity of the control assay.

Assays were performed in triplicate with serial dilutions of inhibitor concentrations from 250 µM to 50 nM.

References for Appendix 1

1. Thompson, V. F.; Saldana, S.; Cong, J.; Goll, D. E., A BODIPY Fluorescent Microplate Assay for Measuring Activity of Calpains and Other Proteases. *Analytical Biochemistry* **2000,** 279, (2), 170-178.

Appendix 2.1: 2.13 Eye drop formulation

Dialdehyde **2.13** was formulated into an eye drop of the following composition.

- 0.1% (w/w) compound **2.13**
- 14% EtOH
- 0.9% sodium chloride
- 0.3% hydroxypropyl methyl cellulose
- 0.05% disodium EDTA
- 0.01% benzalkonium chloride
- 84.65% MilliQ purified water

Sodium chloride, hydroxypropyl methyl cellulose, disodium EDTA and benzalkonium chloride were dissolved in a 14% EtOH aqueous solution. Compound **2.13** was added and stirred at rt until a homogenous solution was obtained.

Appendix 2.2: 2.13 intravitreal injection formulation

Dialdehyde **2.13** was dissolved in a 10% ethanol aqueous solution.

Appendix 2.3: 2.13 ointment formulation

Dialdehyde **2.13** was formulated into an ointment of the following composition;

- 1% compound **2.13**
- 25% cetyl stearyl alcohol
- 35% lanolin
- 39% paraffin oil.

The cetyl stearyl alcohol, lanolin and paraffin oil were melted at 50^{o}C to give a light orange solution. Compound **2.13** was added and the mixture stirred at 50^{o}C until a homogenous

solution was obtained. This was cooled to room temperature and measured into the application syringes.

Appendix 2.4: 5.14 ointment formulation

Aldehyde **5.14** was formulated into an ointment of the following composition;

- 1% compound **5.14**
- 25% cetyl stearyl alcohol
- 35% lanolin
- 39% paraffin oil

Compound **5.14** was dissolved in cetyl stearyl alcohol at 50^{o}C. Lanolin and paraffin oil were then added. The mixture was stirred at 50^{o}C until a homogenous solution was obtained. This was cooled to room temperature and measured into the application syringes.